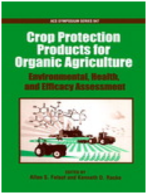

Crop Protection Products for Organic Agriculture

Environmental, Health, and Efficacy Assessment

EDITED BY

Allen S. Felsot and Kenneth D. Racke

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Crop Protection Products for Organic Agriculture

Environmental, Health, and Efficacy Assessment

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Sponsored by the
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Foreword

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Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

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Preface

The U.S. Department of Agriculture (USDA) National Agricultural Statistics Service documented a 63% increase between 1995 and 2003 in the number of farms certified for organic production practices, although the total acreage (2.2 million acres) remains small by comparison to non-certified farms (794 million acres). Certification of a farm operation as organic follows the administrative rules promulgated as 7 CFR Part 205, the National Organic Program (NOP). Administered by the USDA Agricultural Marketing Service, the NOP defines organic production as "A production system that is managed in accordance with the Act and regulations in this part to respond to site-specific conditions by inte-grating cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity." A distinguishing feature of certified organic production is the requirement to operate under a system plan that must be audited by a certifying agent. In contrast to popular perception, this plan can include the use of pesticides for crop protection although philosophically they may be used as a tool of last resort. Given that integrated pest management (IPM) theory promotes the judicious use of pesticides as part of a comprehensive approach in combination with other tactics, the differences between so-called conventional crop protection practices that rigorously follow IPM and certified organic practices may be defined more by the types of pesticides deployed rather than by "use versus no use".

All pesticides, regardless of origin (synthetic or natural) or organic certification status, must undergo regulatory evaluation as part of the Environmental Protection Agency (EPA) registration approval process. Therefore, we felt it was time to publicly explore from a scientific perspective what is known about products suitable for organic crop production. We considered also that such products would likely fall under the rubric of EPA's biopesticide and/or reduced risk programs, although pesticides with either status may not meet the requirements allowing use in organic production.

As a first step in understanding the crop protection products used in organic systems, and by extension more compatible with the ideals of IPM systems, we organized in the spring of 2003 a multi-day symposium titled, "Environmental, Health, and Efficacy Aspects of Biologically Derived and Certified Organic Pesticides". This symposium was part of the technical program of the American Chemical Society's (ACS) Division of Agrochemicals. Some observers may consider it odd that the ACS would sponsor a symposium about seemingly "non-chemical" agricultural technology that benefits organic farmers. However, the Division has long convened national programs examining all aspects of pest control technology. Subject matter has been interdisciplinary ranging from basic chemistry and biochemistry to biology and applied ecology. The perspective has encompassed many scales of measurement ranging from the laboratory to the field and beyond to the landscape, region, and global system. The scientific aspects of risk assessment of crop protection technologies as well as the societal mandates of risk management are subjects of great interest.

Perceiving many misperceptions about the permissible technologies used to protect crops under certified organic production practices, we had decided it was time to scrutinize the tools as we would any other chemical technology. Our purpose was not a cynical position but essentially an airing of questions that are asked before any pesticide is registered regardless of the type of production system it will be deployed in. Given that the scientific literature seemed to lack significant health, environmental, or efficacy assessment information for many crop protection products deemed suitable for organic agriculture, we invited the symposium participants to write a chapter for this book that we hoped would help meet this need.

One of the primary goals in assembling the chapter subject matter for this book has been to stimulate further research into new pesticide technologies that will be acceptable to the organic agricultural community but also likely to be adopted by non-organic producers. The new products will necessarily have a reasonable certainty of causing no ecological or environmental harm, but will also have high efficacy without harming native or introduced biological control organisms. In this volume, Chapter one provides a brief overview of the needs for crop

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protection and the evolution of strategies for attaining technological competence that are compatible with environmental stewardship. The next set of chapters detail how risk assessment of pesticide technologies is handled from the perspective of the agrochemical industry and by the governing bodies for organic agriculture. The remainder of the book examines specific pesticide technologies compatible (or already approved) for organic agricultural production and attempts to make transparent the information in hand regarding human and ecological safety as well as efficacy.

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Chapter 1

Chemical Pest Control Technology: Benefits, Disadvantages, and Continuing Roles in Crop Production Systems

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In the U.S., certified organic agriculture has been clearly demarcated from conventional agriculture by regulatory rules under the National Organic Program. However, neither organic nor conventional agriculture has a clear scientific definition. From a public perspective, organic agriculture is often defined simplistically and contrasted with conventional agriculture by the non-use of pesticides. Furthermore, organic agriculture is often viewed as the epitome of sustainability. In reality, sustainable practices are not well defined because systems are dynamic and practices must be constantly adapting within the context of changing biotic and abiotic characteristics of a field or landscape. Sustainability is a concept best viewed as a goal of all growers, whether they associate themselves with the terms organic or conventional. Also, practices deployed by organic growers for pest control are increasingly practiced by conventional growers. Examples include the use of crop rotation for controlling soil borne pests (such as the corn rootworms, nematodes, and fungal diseases) and the use of pheromones for mating disruption of moths, especially in tree fruit. Pheromones are but one type of pesticide registered by the EPA and approved for use in certified organic agriculture. Others include certain formulations of spinosad and azadirachtin and certain

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microbial pesticides based on non-genetically engineered cultures of *Bacillus thuringiensis* among other species. Considering that many agrichemical companies are now trying to develop and market EPA-approved reduced risk pesticides, a convergence in techniques may be taking place among all types of growers as they seek to manage the same problems consistent with a desire for environmental stewardship. Rather than further differentiate between organic and so-called conventional practices, this chapter seeks to understand common reasons for why growers use pesticides, the advantages and disadvantages of pesticides, and the continuing role of pesticides in crop protection regardless of agronomic practices. Over reliance on singular techniques in any agronomic system may lead to their eventual failure. To be sustainable, pest control must be conducted using the principles of integrated pest management (IPM) as a decision support system. The IPM strategy is based on a confluence of biological and economic information and is implemented by integrating multiple compatible control tactics.

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The tools used to sustain a thriving agricultural enterprise are often viewed as the demarcating characteristic of different types of management systems. This perspective relegates any discussion of food production systems to a black or white perspective, best characterized by the cliché—pesticides bad, no pesticides good. Any production system using pesticides has been labeled as conventional and not viewed as sustainable. Furthermore, organic agriculture has become identified with "sustainable" agriculture. The reality is that all production techniques use pesticides or at least rely on the potential to use them. The difference among production systems is not the dichotomy of use but the specific types of products that are used.

Unfortunately, the negative or favorable public opinions about agricultural practices based mainly on pesticide use (or non-use) hinder the understanding of agricultural impacts in a broader context and the convergence of all production systems toward sustainability. Integrated farm management seems to be the objective of all farming systems and specific practices are blurring among the different types of management schemes. For example, so-called conventional pome fruit (e.g., apple and pear) growers are using pheromone-based mating disruption techniques for controlling codling moth injury, but they still use cover sprays of organophosphorus insecticides and more increasingly reduced risk chloronicotinyls. Is this practice conventional or an alternative integrated technique in transition to a greater probability of future productivity? Similarity in practices also applies to soil management. Organic growers formerly relied heavily on tillage (cultural practices) for weed control, but have switched to reduced and no tillage practices that "conventional" growers in the Corn Belt adopted in great numbers over 20 years ago.

Crop protection chemicals play a continuing role in modern agriculture, regardless of what techniques are deployed or how a commodity is marketed. One could even argue that plant breeding for antibiosis factors adversely affecting pest physiology is just a different strategy for deploying a chemical to aid crop protection. Nevertheless, to promote an understanding of the role of crop protection chemicals, this chapter provides a perspective on why growers use these tools, what advantages they confer, and a reckoning of their disadvantages. Necessarily, this chapter must cover some historical aspects of the evolution from integrated control to integrated pest management and the evolution of strategies for attaining technological competence that is compatible with environmental stewardship. Finally, issues associated with crop protection chemicals are comparatively examined in so-called conventional and certified organic production systems.

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Why Producers Use Crop Protection Chemicals

Numerous organic production advocacy websites, as well as refereed journal papers and review articles agree on one principle—optimizing agricultural productivity requires a crop protection technology. Certain agronomic practices (for example through the use of crop rotation, polycultures, host-plant resistance) achieve adequate control of some pests and are adaptable to organic and non-organic production. Often, however, agronomic practices by themselves are insufficient to obtain economically successful production, especially in the high-value per acre production of vegetables and fruit. Since the early 20th century inorganic and then later synthetic organic pesticides have closed the gap in pest control. But pesticides had been used since antiquity (*1*, *2*). The key to understanding the need for management and a stopgap when agronomic techniques alone are inadequate lies in a comparison of natural and agricultural ecosystems (agroecosystems).

Natural ecosystems are self-sustaining by virtue of their biotic diversity, having shaped and been shaped by the abiotic environment. The soil stores plant nutrients, which are continually recycled throughout components of the ecosystem. A combination of biotic diversity and soil fertility allows the system to respond to perturbations and re-establish its productivity. A good example of a natural ecosystem's ability to quickly re-establish productivity on a landscape and move through successional stages is the area around the Mt. St. Helens Volcano in Washington State. The catastrophic explosion of Mt. St. Helens in 1980 devastated a landscape that could be analogized to the destruction wrought by a nuclear bomb explosion. However, life reemerged in the vicinity and nearby affected areas of the volcano, proving the resilience of natural ecosystems to regenerate after perturbations. The lesson of Mt. St. Helens is that the biotic components of ecosystems may constantly change but natural ecosystems function as in a steady state and stable associations of species eventually develop. Such stability is called the climax state (*3*).

Agroecosystems in contrast have significantly less biotic diversity than natural systems by virtue of the need to maximize production of a single species (*4*). Nutrients, especially nitrogenous forms, are annually removed from the agricultural system rather than recycled. The soil of field crops (corn, soybean, wheat, cotton, etc.) is continually disturbed and much of the biotic component must re-establish on an annual cycle. Orchards and other perennial crops lack wholesale annual disturbances but the biotic diversity is very limited and pollinators have to be imported to produce a crop. When diversity is lacking, the populations of just a few species can explode to dominate the system. When those species compete with us, they are pests needing control.

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Pests that become established in our agroecosystems may be either native to the ecosystems that the crops have replaced or they may have been accidentally imported from other countries (*5*). In the former case, native pest organisms (e.g., insects, plant pathogens) have a readily abundant food supply and populations can quickly increase if the abiotic components (like weather) are favorable. Disturbed soil also provides opportunities for weeds to out compete crops. Native pests do have natural biotic regulatory factors (for example natural enemies like parasitoids, predators, and pathogens) but the lack of biotic diversity and frequent system perturbations can make these factors insufficient by themselves to prevent economic losses of crop yield. Imported (or exotic) pests, especially originating from places of similar latitude to the destination fields, experience conditions that allow them to thrive easily because their biotic regulatory factors are often missing or their other natural mortality factors are not operational.

The conflict between the economic value of a crop and its susceptibility to damage from an explosive pest population demands the need for management of both the crop and the pest. The continual removal of nutrients from the soil by harvesting a crop and the comparatively short time interval between successive plantings necessitates the addition of readily available nutrients. In short, the structure and characteristics of an agricultural ecosystem and pest population ecology necessitate a high level of management and inputs to maintain soil fertility and protect the production of the harvestable seeds, fruit, and vegetation.

Benefits of Crop Protection Chemicals

Economic and Environmental Benefits

Pesticide and fertilizer use has been recorded since ancient times, suggesting that ecosystem management is not a recent cultural attribute. In the context of production agriculture, the objectives of pesticide use are to increase production efficiency and yields; reduce cost of food and especially increase availability of fruits and vegetables; improve food quality and losses during transport and storage; improve soil conservation; and ensure a stable and predictable food supply (*2*).

Pesticide use is widespread on farms, but more importantly, different classes of pesticides are differentially used (*2, 6*), suggesting that growers make decisions based on need rather than solely on prophylaxis. For example, during 2002 approximately 303 million acres of crops were harvested, and 95% were treated with some type of pesticide. However, 64% of the acreage was treated to control weeds (i.e., herbicide use), 22% to control insects (insecticide use), 6% to control diseases and nematodes (fungicide and nematicide use). Another 4% of the crop acreage was treated with a plant growth regulator for fruit thinning, growth control, or defoliation.

The intensity of specific pesticide classes also varies significantly by crop. Grains tend to be disproportionately treated with herbicides, but fruit and vegetables mostly receive insecticide and fungicide applications (Table I).

The benefits of crop protection chemicals for improving and protecting crop productivity is difficult to separate from the effects of hybrid seed technology and other plant breeding advances. Nevertheless, an examination of crop yields relative to land under production shows both types of technologies have had major contributions. For example, the greatest proportion of U.S. farmland is devoted to corn production. A historical examination of area of land, yields, and the introduction of different technologies over time suggests that insect control (mainly of the corn rootworm complex) has greatly enhanced the effectiveness of hybrid seed technology (Figure 1). Furthermore, the introduction of modern synthetic herbicides facilitated widespread adoption of conservation tillage in the Corn Belt that greatly reduced the number one problem of agriculture—soil erosion and sedimentation in rivers.

Perhaps an even more compelling case for the role of crop protection chemicals, especially fungicides and fumigants, in crop production efficiency is suggested by potato production statistics. In 1900 nearly 3 million acres of potatoes were harvested yielding an average of 52 cwt/acre (*10*). In 1950, average yields were 153 cwt/acre. In crop year 2004, 1.2 million acres of harvested potatoes yielded an average 752 cwt/acre. Surely advances in plant breeding play an important role in production increases but by the 1950's fumigants for control of nematodes became widely available nearly coincidentally with the widespread adoption of mineralized fertilizers. But the production trends strongly suggest an environmental benefit in that only 40% of the total potato acreage planted in 1900 could produce in 2004 seven times more potatoes.

The aggregate economic benefits associated with pesticide use have been subjected to various empirical modeling exercises and expressed as the loss of production if pesticides were not used (*2*). Production losses during the mid-

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Table I. Percentage Use of Pesticide Classes on Major Crops During Crop Years 2003 or 2004 (*7, 8*)

Crop	*Herbicide*	*Insecticide*	*Fungicide*
Corn	95	29	<1
Soybean	97	4	1
Wheat	45	7	2
Cotton	98	64	7
Potato	91	84	91
Apple	42	94	90

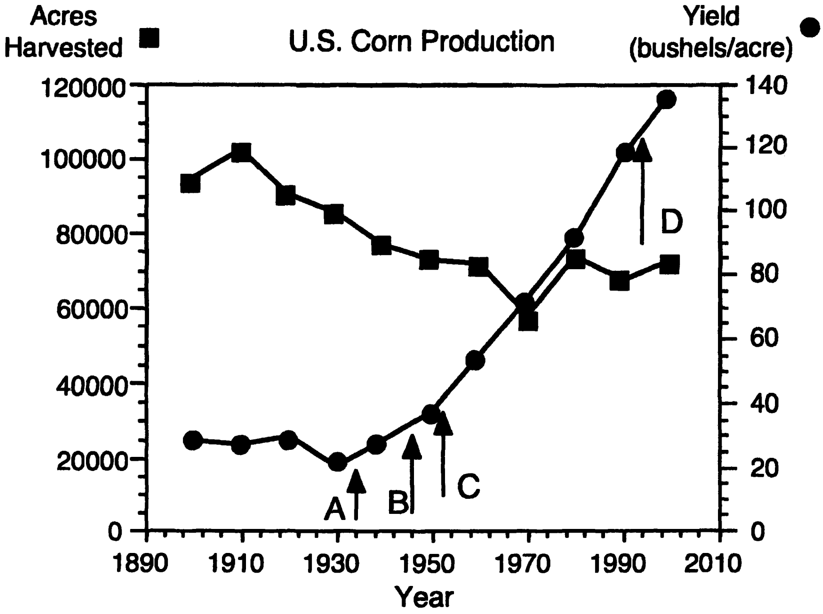

Figure 1. Historical trend in U.S. corn production and approximate timeline for introduction of crop production technologies. A: hybrid seeds; B: mineralized fertilizers; C: soil insecticides; D. transgenic crops (9).

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1980s were estimated to be as high as 37% of total output (*11*). This estimated loss occurred despite pesticide use, but the estimate seems rather high when assessed against specific crops analyses. For example, one of the most destructive pests of potatoes, late blight disease, broke out in the Columbia Basin of Washington State and Oregon during 1995. Fungicide use rose from typically 2 applications per season to as many as 12 (*12*). However, yield differences between the pre and post blight outbreak were only 4-6%. On the other hand, without any management, the blight epidemic could have reduced yields 30-100%.

Other economic analyses have projected the effect on fresh and processed vegetable and fruit yields if pesticide use was reduced 50% or simply not used at all (*13*). Reductions in fresh fruit yield were 40% and 75%, respectively. Another study estimated that a total pesticide use ban would require an additional 2.5 million acres of vegetable and fruit production to make up for the yield loss (*14*). Although modeling estimates of the impacts of decreases in pesticide use or no use seem large, the actual decrease in yields (and consequent effects on consumer prices) would depend on the availability of other alternative crop protection technologies or practices (*2*). For example, field crops like corn can be grown with minimal herbicide use if tillage is used more frequently. Of course, such an action would counter the benefits of reduced tillage for soil conservation. Vegetable and fruit production, however, would likely be most adversely affected by wholesale loss of use of insecticides and fungicides owing to the disproportional problems with insect pests and plant pathogens. Loss of pesticide availability would also have adverse impacts on consumer prices and potentially loss of domestic sources of supply as production is dislocated to other regions (*15*).

In summary, economic analyses of various kinds seem in agreement that pesticide use has been definitely associated with profitable returns to farmers (and thus to society). The quantification of such returns is problematic owing to a lack of large scale empirical testing. Furthermore, global percentage loss predictions mire the unique agroecology of local and regional landscapes and thus tend to skew perspectives of the benefits and/or costs of pesticide use.

Practical Advantages of Crop Protection Chemicals

In addition to their economic benefits accruing from the objectives for which they are used, pesticides have certain advantages for crop protection (as well as production) that make them very convenient, efficient, and cost-effective (*4*). First, for most cropping systems and in some cases-insect vectored diseases, pesticides are the only practical available technology because other technologies are not available, unproved, or do not work efficiently. For example, hybrids of certain crops may lack a pest-resistant cultivar. In other cases, a non-chemical pest control practice fails to work over time. An example of the latter situation is the apparent adaptation of western corn rootworms to the practice of annual corn-soybean rotations that were very successful in reducing the need for soil insecticides (*16, 17*).

Second, pesticides have rapid curative action in preventing loss of crop yield or protecting human and animal health. Thus, they can be used when a pest population becomes intolerable. One of the tenets of integrated pest management (IPM) discussed below is eschewing prophylactic sprays in favor of "as needed" treatments. Thus, there may be a very short time window in which the pest needs to be controlled and non-chemical methods may lack a rapid enough action.

Third, the diversity of locations where crops are grown means different pest complexes thrive under a wide range of climatic conditions. Pesticides have a wide range of properties, uses, and methods of application that can cover many problems as they arise. The inorganic pesticides used during the first half of the 20th century and the first wave of synthesized pesticides after 1950 were generally broad spectrum but not necessarily adequate for all cropping systems. Over the last thirty years new chemistries have been introduced to narrow the spectrum of activity. Along with new formulations and application methods, modern pesticides can be better tailored to specific crop pest problems. Relatedly, insecticides introduced over the last 15 years are also much less toxic to the natural biocontrol organisms than the broad-spectrum synthetics introduced during the 1950s. Furthermore, modern pesticides are rapidly biodegradable in the environment and do not bioaccumulate in lipid tissues as did the chlorinated hydrocarbon and cyclodiene pesticides that were heavily used prior to their ban in the early 1970s.

Finally, the economic return-cost ratio for pesticide use is generally favorable. The ratio depends on the specific crop because the annual commodity price must be factored in as well as the site-specific yield and expenses due to chemical purchases. Nevertheless older estimates for return ranged from \$4-\$29 for every \$1 spent (*4*) and more recent estimates suggest a \$3-\$6 rate of return per \$1 spent (*15*). Pertinently for the grower is the comparatively low incremental cost of pesticide use relative to all production expenses. The most recent estimate (crop year 2002) is that purchase of pesticides represents 4.4% of total expenses compared to the 12.7% of expenses for hired and contract farm labor (*18*). Pesticides themselves help lower costs by substituting for labor. For example, fruit thinning required in the pome fruit industry is mostly done by chemical thinners but still requires some hand thinning if loads are still deemed excessive. Organic lettuce growers rely on hand weeding to attain profitable production and have petitioned the State of California against stricter labor rules, ostensibly because adequately efficient approved herbicides are unavailable (*19*). Thus, the lack of appropriately available pesticides adds to labor costs.

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Disadvantages of Crop Protection Chemicals

Ever since the publication of Silent Spring (*20*), pesticide disadvantages with respect to environmental and human health have been at the forefront of public discussions. From the perspective of agricultural productivity, however, chemical crop protection technologies have two main disadvantages. Perhaps the most important is evolution of pest resistance as frequency of genes conferring susceptibility is reduced in successive generations (*2, 21*). The second disadvantage occurs when broad-spectrum insecticides are used causing within field loss of abundance in insect natural enemies (i.e., generalist predators and host-specific parasitoids). Loss of these natural biotic population mortality factors can give rise to secondary pest outbreaks as well as resurgence of the pest a grower originally intended to control (*5, 22*).

Much uncertainty remains today about effects of pesticides on human health. However, an improved perspective on the problem should emerge if human health is bifurcated into worker and consumer exposure-associated hazards and risks. The former group includes pesticide applicators (including handlers, mixers and loaders along with applicators) and post-application workers (e.g., thinners, irrigators, harvesters). Even this group can be subdivided by exposure with the handlers receiving greater dermal and inhalational exposures than post-application workers. Several states, e.g. California (*23*) and Washington (*24*), maintain incident databases recording workers claiming an effect from pesticide exposure. Because the illnesses occur within short time intervals of the exposure, the cases within the databases represent acute toxicity incidents.

Chronic effects related to neurological impairment and/or cancers not associated with an acute exposure in workers have been suggested in numerous epidemiological studies (*25*). Unfortunately, almost none of the epidemiological studies published to date have measured pesticide exposure directly, making the observed dose-response relationships invalid. A case in point is the federally funded Agricultural Health Study (AHS) that has been intensely focusing on worker epidemiology related to pesticide exposure in two very large cohorts of farmers in Iowa and North Carolina (*26*). Several published studies from the AHS suggest small elevated relative risks for cancer and perhaps neurological effects that exhibit a dose-response relationship (*27, 28*). However, an early AHS associated publication showed that the program's exposure metric of farmer self-reported pesticide use surveys may be unreliable. In short, two surveys about pesticide use given one year apart to pesticide users showed only 50-60% agreement for duration, frequency, or decade of first use of specific pesticides (*29*).

Consumers may be exposed to pesticide residues via food, water, and residential use. However, the levels of residues are quite low compared to what workers experience. Other than the rare accidental acute exposure leading to

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acute toxicity, evidence is weak that the chronic toxicity effects observed in rodent testing experiments occurs in the general population. Nevertheless, positive results for chronic effects (including tumors, immune and endocrine system dysregulation, or other systemic pathologies) in rodent studies maintain the continuing uncertainty over the effects of pesticide residue exposure. Lost in the conjecture about the effects of environmental exposures is that the rodent studies on pesticides re-registered over the last decade are all done at non-environmentally relevant doses (i.e., the exposure rates far exceed environmental levels) (*24*). Also, the exposure frequency is constant through the lifetime equivalent of the rodents but are much more likely to be intermittent to humans. This likelihood was illustrated by comparing estimates for organophosphorus insecticide exposures based on biomonitoring and EPA aggregate assessments calculated from environmental residues (*30*).

Uncertainty about the potential for adverse ecological effects of pesticides has its legacy in the physicochemical properties and biological recalcitrance to metabolism of DDT metabolites (mainly DDE) and chlorinated cyclodiene insecticide residues. The high rates and widespread use of these long-banned compounds during the 1950s and 1960s led to ubiquitous residues that could bioaccumulate within terrestrial and aquatic food webs (*31*). Although DDE itself is not particularly toxic to birds following single or limited exposures, continual bioconcentration especially in central nervous system tissue was diagnosed as a causal mortality factor in predatory birds. A few researchers carried out monitoring experiments that correlated DDE levels in eggshells and the putatively reduced thickness of those shells compared to museum specimens (*32, 33*). This research led to perhaps the first ecologically relevant chronic effect—namely, DDE caused adverse reproductive effects.

The gradual and finally complete replacement of the chlorinated hydrocarbon insecticides with organophosphate ester (OP) chemistries during the 1970s achieved the desired rapid environmental degradation of insecticide residues and no tissue bioaccumulation. However, the OP insecticides remain the most toxic of pesticides to terrestrial and aquatic fauna. OP insecticides are gradually being phased out and replaced by pyrethroid insecticides. The transition has already been completed in urban environments. Pyrethroid insecticides have very low toxicity to terrestrial organisms but fish and aquatic invertebrates are highly susceptible to residues of low parts per billion, as well as residues accumulating in sediments (*34, 35*).

Recently registered pesticides of the chloronicotinyl class and the new insect hormone agonists (acyl hydrazines) have very low toxicity to non-target terrestrial and aquatic fauna. However, one chloronicotinyl, imidacloprid, has been subject to farmer's complaints about bee kills when it is used as a seed-applied systemic (*36*). Indeed, the compound seems to have extraordinarily high toxicity to bees (*37*). On the other hand, other research results have challenged whether imidacloprid residues are truly sufficient to have caused the alleged bee kill incidents (*38, 39*).

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Finally, a problem with agricultural reliance on chemical technologies is the impediment to future product development and availability engendered by the high research, development, and marketing costs. Owing to the plethora of required human and ecological tests, the costs of bringing a chemical to commercial fruition are approaching $200 million for synthetic organic chemicals. Furthermore, even reduced risk chemicals, whether synthetic or biologically derived, face very high costs because the testing requirements are the same. The return to the pesticide registrant is not realized until well into 10 years of the patent life (*40*). However, pesticides considered reduced risk might have the advantage of being placed on an EPA fast track assessment.

The Continuing Role for Crop Protection Chemicals

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During and immediately after World War II, DDT was significantly beneficial in reducing the incidence of vector-borne diseases. When formulated as a dust it could be directly doused on people for control of lice, protecting soldiers and European citizens from lice-borne typhus. DDT has low acute toxicity and thus could be handled safely without adverse reaction when applied directly on skin. Countries with endemic malaria, including the post war U.S., quickly deployed DDT against mosquitoes and witnessed malaria incidence drop quickly (*41*). By 1950, DDT was widely adopted for crop protection and began to replace a reliance on inorganic arsenicals for insect control, especially on tree fruit and vegetable farms. DDT was quickly joined in the insecticide arsenal by introduction during the early 1950s of the chlorinated cyclodienes (aldrin; heptachlor) and chlorinated hydrocarbons (lindane) that became widely used in corn production for control of the soil dwelling insects like corn rootworms and wireworms.

Not long after the widespread commercialization of DDT, entomologists noted pest control problems that belied the reputation of DDT as the silver bullet. Insect resistance to DDT quickly evolved. Secondary pests rose to prominence as the populations of endemic natural enemies were decimated by DDT's broad spectrum of insecticidal activity. The slow recovery of the natural enemy populations led to resurgence of the primary pests. Although DDT was still considered to not pose any toxicological problems, its bioconcentration in fatty tissue including cow's milk had already been revealed by 1945 (*42, 43*). In the early 1950s, research began to show that toxic concentrations of residues could move into aquatic systems (*44, 45*). Indeed, Rachel Carson had relied on the growing literature already published prior to 1960 to voice alarm in *Silent Spring*.

In 1959, entomologists at the University of California at Riverside wrote a seminal paper that laid out the problems with sole reliance on chemical control technology (*5*). They did not completely eschew pesticides but argued persuasively that chemical control had to be integrated with non-chemical control strategies. Thus was born the integrated control concept that became the forerunner of the integrated pest management (IPM) strategy.

The integrated control concept recognized that pest control was most efficient when biological control (i.e., natural enemies) and cultural practices were integrated with chemical control. For integrated control to be effective over the long-term and environmentally safe, however, the insecticides used had to have certain characteristics and be deployed judiciously. Selectivity (i.e., high toxicity to pests; low toxicity to nontarget organism) was the key characteristic necessary to successfully integrate chemical and non-chemical control techniques. Selective insecticides should only be applied when the ratio of pests to natural enemies was unfavorable for adequate crop protection. Pesticides must degrade rapidly and not leave residues that would expose biological control agents once the pest population was knocked down. Finally, the integrated control concept pushed for the development of insect pathogens as insecticides.

Implementation of the integrated control strategy was to be based on an understanding of population ecology. Population density was known to fluctuate about some general equilibrium position, and the pest manager's role was to understand the population level that could cause economic injury. The economic injury level (EIL) is the pest population density causing sufficient crop damage to prevent economic return from exceeding costs of production. To prevent economic losses control techniques were to be implemented at a population level called the economic threshold that was lower than the population density associated with economic injury. The economic threshold (ET) is the pest population level at which control measures would be implemented to prevent the population from reaching the economic injury level. Theoretically, at the ET the cost of control would equal the return from implementing the control.

Integrated control during the 1960s evolved more formally into integrated pest management and eventually the concepts were expanded from insect pest control to disease and vegetation management. IPM has taken on many definitions but has been comprehensively described as "a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment" (*46*). This definition suggests the ecological and economic concepts that gird IPM as well as includes some perspective that risk assessment and management are integrated. Furthermore, the definition shows that IPM considers all stakeholders beyond just the growers' needs.

IPM programs have three objectives (*47*): maintain profitability when managing pests by taking actions only when economically justified; minimize selection pressure on pest populations from the chosen management tactics to allay resistance development; maintain environmental quality by minimizing the impact of management tactics on the environment.

Neither the conceptual definition of IPM nor the stated objectives of an IPM program preclude the use of chemical control tactics. Rather all control tactics

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are relegated to the position of "tools" that should be compatibly integrated with one another. Before opening the toolbox, however, IPM principles involve the following elements.

- Correct taxonomic identification of the pest (and thus its biology);
- Characterization of population dynamics and mortality factors;
- Development of pest sampling and scouting (monitoring) plans;
- Characterization of economic injury levels and development of economic thresholds;
- Development of alternative control options including biological control (either through conservation of natural enemies or their importation and release); cultural control (e.g., crop rotation; trap crops); mechanical control (e.g., weed cultivation; vacuum systems to suck up soft-bodied insects); breeding host-plant resistance; chemical tactics (judicious application of selective pesticides, including mating disruption using pheromones).

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A realistic appraisal of IPM programs as they are currently practiced on the farm (as opposed to university research efforts) reveals that pesticides are still the primarily deployed control tactic. However, crop consultants play an important role in carrying out population monitoring and making recommendations to growers, especially in the tree fruit and vegetable industry. Nevertheless, the reliance on pesticides (despite their previously stated disadvantages) stimulated the call for IPM to evolve to EBPM (ecologically based pest management) (*22*). Pesticides would not eliminated from consideration under an EBPM strategy but the central theme of pest management programs should center around biologically based control, not chemical control.

Crop Protection Chemicals for Conventional, Organic, and Sustainable Production Systems: Choose Your Poison

A growing number of books and journal articles are deconstructing organic agricultural production techniques, characterizing sustainable practices, and comparing both farming strategies to conventional techniques. Unfortunately, the terms conventional and sustainable lack any scientifically based definition, while organic production has been socio-politically codified in a Federally backed slate of rules and regulations known as the National Organic Program (NOP). Furthermore, agricultural practices can change rapidly depending on the availability of new tools and techniques. The idea that techniques are continually evolving based on trial and error is applicable to determining whether practices are sustainable. Indeed, agricultural sustainability is probably best considered a goal to be achieved rather than a prescriptive set of practices.

Organic agriculture is bound by the suite of government rules necessary to achieve certification. The latter statement should not be taken as impugnment of organic agriculture but rather an argument that the real difference among production systems is governed by a set of standard operating procedures either explicitly stated as in the NOP or as amorphously practiced by those outside of the organic growers community. Certainly, individual organic growers are also eager for data from experiments on production techniques that are compatible with NOP rules as well as compatible with the goal of sustainability.

Much of the literature is in agreement that sustaining agriculture relies on high quality soil parameters, characterized for example as adequate soil organic carbon, soil moisture holding capacity, and diversity of microbial function. The tools for achieving optimal soil fertility simply solve the problem of "nitrogen deficit", i.e., how much nitrogen in mineralized or organic form should be added to a crop given information about its nitrogen needs. Tillage can be altered to promote long-term build up of organic carbon residues. Common techniques are available to and used by organic and non-organic producers. Both styles of agriculture should adhere to practices that best reduce soil erosion.

For crop protection, however, similar agreement as to what constitutes safe and effective pest control tools is lacking, although all production practices would claim to adhere to the principles of IPM. Organic growers by rule cannot use synthetic materials unless approved by the National Organic Standards Board (NOSB). But no pesticide (NOSB-approved or otherwise) can be used unless vetted by the EPA. All growers to a greater or lesser extent use similar non-pesticidal techniques, such as augmentation of natural enemies to improve natural biocontrol mechanisms or crop rotation to eliminate pest hosts for one generation. But unlike manure, which is available "off-the-shelf" and can be applied as needed, biocontrol and cultural control must be strategically enhanced within a system of production.

Some pesticides will be compatible with biocontrol and some will not. NOSB approval is no guarantee for compatibility nor is the mere fact of EPA registration. Agricultural systems are just too diverse to generalize. However, the human and environmental safety and efficacy of all pesticides can be subjected to scientific assessment prior to their approval (whether by EPA alone or additionally by the NOSB). Whereas the majority of synthetic pesticides have been well studied long after their approval, and adjustments in permissible uses or addition of new uses made in response to new information, the pesticides approved for certified organic production have been comparatively ignored.

Perhaps the most popular conception among consumers of organic foods is that they lack pesticide residues and other additives. The basis for this belief is the often-repeated argument that organic agriculture distinguishes itself from conventional production methods because no synthetic pesticides are used. Prolonged pronouncements of no synthetic pesticide use easily evolve into a perception of no pesticide use. Contraction of no synthetics use to the equivalency of no use at all may be facilitated by the myth that somehow synthetic substances are generically different in obeisance to thermodynamic laws and reactivity than natural substances.

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The reality is that analytical surveys of organic commodities reveal that they contain synthetic pesticide residues both banned and currently registered, albeit at much less frequency than so-called conventional foods (*48*). However, the organic producer is "victim" because the residues have not resulted from willful use but are more likely inadvertent due to airborne transport and deposition as well as soil-borne from past use. Recognizing the ubiquity and mobility of environmental residues, NOP rules allow inadvertent pesticide residues up to 5% of the established Federal tolerance level without a loss of organic certification.

On the other hand U.S. rules for certification of organic production allow for the willful use of approved crop protection products. Under the Federal Insecticide Fungicide and Insecticide Act (FIFRA) many of theses products are legally pesticides and must be registered with the EPA. Later chapters in this book provide details about some of these products (e.g., the pheromone codlemone, the organic formulations of spinosad, various forms of neem), and are examined from a similar perspective as the synthetic pesticides.

Although organic production practices have been simplistically (and probably unfairly) condensed to no pesticide use, common techniques of pest control are shared with non-organic producers. For example, many tree fruit producers currently use pheromones to disrupt pest mating and the microbially derived active ingredients called spinosyns because they have proven advantages. Adoption of IPM principles as a decision support system focuses attention on conservation of natural enemies of insect pests, especially in high value vegetable and fruit production. This objective is equally shared by the spectrum of production systems. Organic producers tout crop rotations as cultural control techniques for disrupting annual insect pest infestations, but conventional corn and soybean growers have routinely used such practices. In short, techniques will increasingly become common among all types of producers when they have proven benefits.

Organic and non-organic growers face similar problems as well as benefits from pest control decisions. For example, organic growers have already witnessed evolution of insect resistance to sprays of *Bacillus thuringiensis* (*49*), a problem long faced by conventional agriculture's reliance on synthetic pesticides. Observations in parts of the Midwest of a western corn rootworm variant that exhibits a behavioral resistance to multiple crop rotations (*50*) show control tactics are not really permanently sustainable in dynamic systems with adaptable pests. Pest adaptability raises a warning against over reliance on one technique (as opposed to an integration of methods). Such over reliance may be a causal factor leading to microbial adaptation for rapid breakdown of allylisothiocyanate allelochemicals produced when growers cover crop potato fields with certain mustard species to control nematode and insect larvae (*51*).

In conclusion, agricultural producers of all types share common problems in pest control but also common solutions. The term sustainable describes a goal that both organic and conventional producers would like to achieve. But organisms evolve in response to selection pressures so practices must also be

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dynamic. All growers need some type of pesticide for pest outbreak emergencies or when the economic damage threshold is so low that no pest can be tolerated. Organic producers use specifically approved products as a last resort, but like non-organic growers using synthetic pesticides approved only by the EPA, they will use them in conjunction with the principles of IPM. Over reliance on any one pest control technique and failure to integrate multiple compatible techniques will lead to pest control problems regardless of the label given to the production system.

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Chapter 2

Crop Protection Product Formulation for the Organic Market

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Organic farmers have been pioneers in the use of alternative methods of crop protection and adoption of reduced-risk pesticides. The growing organic market offers opportunities and challenges for formulators to develop and market products compatible with organic standards. Federal regulations create uniform standards so that formulators are not faced with various state regulations defining organic food and private standards for organic certification. The USDA National Organic Program Rule applies to entire formulations and permits only a limited number of both active and inert ingredients for crop protection in organic production and handling. Compliance verification is another challenge that companies face to enter and profit from this market. The organic market presents research opportunities for product development and commercialization. Formulators who wish to design products for this market have a number of information resources available to provide guidance on regulations, sustainability, and organic principles.

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

Organic food and fiber represents a sector within agriculture distinguished by a set of process-based production standards. Under those standards, organic crops are grown without the use of most synthetic fertilizers and pesticides. Organic livestock are fed organically grown crops and are raised without most synthetic drugs and parasiticides. Organic food is handled without most synthetic substances used in or on the food. The *National List of Allowed Synthetic and Prohibited Non-synthetic substances*, a part of the USDA National Organic Program (NOP) Rule (7 CFR Part 205), contains a limited number of exceptions that are made to this general rule. These exceptions are based on criteria that take into consideration human health and the environment. In all cases, genetically engineered organisms and their products are excluded.

Organic farmers rely on a variety of cultural, physical, mechanical, and biological methods to protect crops from various pests, diseases, and weeds. Organic producers report that they use relatively few substances for pest control, and of those most are used only sparingly in conjunction with other techniques (*1*). Despite the limited use of pesticides by organic farmers, there is a demand for softer pesticides driven by regulatory incentives, farmer preferences to use less toxic means of pest control, and the demand by consumers for organic food with comparable cosmetic quality to conventional at competitive prices.

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Market Summary

Consumer demand has driven the growth of the organic market. Consumers cite a number of reasons why they pay a premium to purchase organic food, with reduced risk from exposure to pesticides as one common reason cited (*2*,*3*). Organic food is less likely to contain pesticides than conventional food, less likely to contain multiple residues of pesticides, and when contaminated will in general have lower levels of pesticides than conventional food (*4*). Empirical evidence suggests that such reduced exposure results in lower residual levels in humans who consume organically produced food (*5*).

The EPA establishes limits on pesticide residues in food based on risk assessments (40 CFR 180). Food is excluded from being sold as organic if it exceeds 5% of EPA tolerances for any pesticide prohibited in organic production (7 CFR 205.671). Most pesticides allowed in organic production are subject to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 7 USC 136 *et seq.*) and have undergone another review process under the USDA organic program that takes into account the impact of the substance on human health and the environment. As a result, most pesticides used in organic production are exempted from the requirement of a tolerance.

Consumers are willing to pay a premium for organic food. According to figures from the USDA, sales of organic food were approximately $7.8 billion when the NOP Rule was published in December 2000. In 2001, nearly 7,000 farms managed over 7.5 million certified organic acres (*6*). By 2002, organic food had grown to an estimated $9.35 billion in the United States (*7,8*). The organic market has been growing in the double-digits for over ten years, with overall growth forecast to continue in the 10-20% range. A worldwide estimate for the year 2000 was $16 billion increasing to $19 billion in 2001, with the U.S. surpassing Germany as the largest single national market for organic food (*9*).

Organic food is now traded in a global marketplace. Much of the organic food sold in the world is not regulated by the USDA. Food produced and consumed in the European Union is subject to the EU Regulation on organic farming (EC 2092/91). Japan has also published a standard for organic food. The International Federation of Organic Agricultural Movements, a non-governmental organization, has developed internationally recognized basic standards for organic production and processing (*10*). Codex Alimentarius of the United Nations has established guidelines for organically produced foods (*11*). Production and consumption of organic food may take place in markets that operate entirely outside of these three major trade zones, and may be subject to either other national regulations or voluntary standards.

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U.S. Organic Regulations

The USDA promulgated the NOP Rule under the statutory authority of Title 21 of the 1990 Farm Bill [PL 101-624], known also as the Organic Foods Production Act (OFPA). The NOP Rule established a Federal standard of identity for food labeled as organic for the first time, moving beyond existing private and state standards [65 *Federal Register* 80548 *et seq.*]. The NOP Rule was the result of a ten-year rulemaking process, with active participation by the organic industry and other interested parties. Broad public opinion, reflected by the largest number of comments that the USDA received up until that time, resulted in an NOP rule that was in general more stringent than the private and state standards that existed prior to implementation. The rule not only banned the products of genetic engineering, irradiation, and the use of sewage sludge, it banned other controversial practices such as the use of antibiotics and application of inert ingredients in pesticides that are not classified as minimum risk.

The authors of OFPA recognized that materials review was an essential part of the functioning of organic standards. Rather than establishing an exhaustive list of what could be used, Congress required that the USDA establish a procedure. Because of advances in science and new information of the environmental and health risks posed by farm inputs, the USDA needed to be able to revise a list of materials based on criteria reflective of the values of the organic community and public participation by all stakeholders. Over time, it was recognized that new materials might be developed that are compatible with a system of sustainable agriculture, and that materials currently being used may need to be prohibited based on new data that show potential risks. With respect to material inputs, all synthetic substances are prohibited, unless they are explicitly allowed on the *National List of Allowed Synthetic Substances* and all natural substances are allowed unless they are explicitly prohibited on the *National List of Prohibited Non-synthetic Substances* [7CFR 205.600- 205.607].

As required by OFPA, the USDA established a National Organic Standards Board (NOSB) responsible, among other things, to recommend materials to the Secretary of Agriculture for inclusion on the *National List.* The USDA established a petition process to submit a request to the NOSB to consider recommending the addition or removal of substances to the *National List.* Petitions must provide detailed information about the material, its source, and environmental impacts. A petition must be filed before a material can be considered either for addition or amendment to the *National List* or for removal. The NOSB also must receive expert consultation from a Technical Advisory Panel (TAP) regarding each material reviewed.

Pest Management Strategies

Organic producers are required by regulation to rely on cultural and biological methods as their first line of defense against pests, weeds, and diseases [7 CFR 205.206(e)]. Crop rotations, beneficial organism releases, selection of resistant varieties, habitat management, and timing of crop production are all important strategies for pest management. Pesticides are used only when biological and cultural methods are insufficient to provide adequate control. A non-exhaustive summary of the various commonly used active ingredients allowed for use in organic agriculture is contained in Table I.

According to a 1999 survey of over 1,000 organic farmers, cultural and classical biological practices such as crop rotation, habitat management, and releases of beneficial organisms are the first lines of defense for most organic farmers. The insecticide that organic farmers use the most when they resort to the application of pesticides is *Bacillus thuringiensis* (Bt), an insect pathogen. Soap is the only other pesticide that a majority of the farmers reported using. These two active ingredients were followed by narrow range oils used to suffocate insects and inhibit fungal growth. The majority of organic farmers do not use botanical insecticides at all. Table II shows that, of the farms responding, only 9% reported frequent use of botanicals (*1*).

The most common practice that organic farmers employ to manage disease pressure is crop rotation (Table III). Cultural practices such as selection of resistant varieties, and management of crop nutrients are other measures that organic farmers use to prevent disease. Most organic farmers do not use fungicides. Of those that do, sulfur and copper are the most widely used.

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Table I. Selected Substances Commonly Used to Protect Organically Produced Crops

Active Substance	*Use / Application*	*Category*
Bacillus thuringiensis	Insecticide	Non-synthetic Biological
Boric Acid	Insecticide	Synthetic
Codling moth granulosis virus	Insecticide	Non-synthetic Biological
Copper products	Algicides, Fungicides	Synthetic
Corn gluten meal	Herbicide	Non-synthetic
Hydrated lime	Fungicide	Synthetic
Hydrogen peroxide	Fungicide	Synthetic
Lime-sulfur	Fungicide, Insecticide	Synthetic
Narrow range (dormant and summer) oils	Insecticide, Fungicide	Synthetic
Neem	Insecticide, Fungicide	Non-synthetic Botanical
Pheromones	Insecticide	Synthetic
Potassium bicarbonate	Fungicide	Synthetic
Pyrethrum	Insecticide	Non-synthetic Botanical
Rotenone	Insecticide	Non-synthetic Botanical
Sabadilla	Insecticide	Non-synthetic Botanical
Soap	Insecticide, Algicide	Synthetic
Spinosad	Insecticide	Non-synthetic Biological
Sulfur	Fungicide, Acaricide	Synthetic
Vitamin D3	Rodenticide	Synthetic

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Weed management was reported to be the greatest production obstacle that organic farmers face (*1*). While specific crop-pest and crop-disease complexes sporadically vex organic farmers, weed management is a universal and continuing problem. In general, herbicides are considered incompatible with organic production. A 1999 survey of organic farmers reported weeds to be the highest priority of organic farmers (*1*). Organic farming systems have traditionally shunned the use of inputs to control weeds, relying instead on cultivation and crop rotation. No-till and minimum till without herbicides offers the best of both worlds—if researchers can make such a system work. Biologically-based weed management has lagged behind biological control of insects and diseases. Various least toxic natural herbicides have limited efficacy, particularly against noxious perennial weeds. Mycoherbicides have some promise but also pose risks to non-target plants. The development of biological and cultural alternatives to herbicides, cultivation, and tillage offers great opportunities for organic farming research.

Table II. Use and Frequency of Pest Management Strategies or Materials (in %) by U.S. Organic Farmers in 1999

Strategy or Material	*Never (%)*	*Rarely or as a Last Resort (%)*	*On Occasion (%)*	*Frequently or Regularly (%)*
Crop rotations	18	1	7	74
Beneficial insect habitat	39	5	18	38
Beneficial vertebrate habitat	60	7	12	21
Bacillus thuringiensis (Bt)	43	12	27	18
Beneficial insect, mite or nematode releases	61	10	18	11
Dormant or summer oils	65	11	13	11
Insecticidal soaps	49	18	23	10
Botanical insecticides (e.g. pyrethrum, rotenone, ryania, sabadilla, quassia, neem)	52	21	18	9
Trap crops	60	13	18	9
Pheromones or mating disruption	78	6	8	8
Viral pathogens (e.g. granulosis virus')	95	3	1	1

NOTE: Based on survey responses of around 1000 to 1100 per category.

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Impacts of the NOP Rule

Initial Impacts

The initial impact of the NOP Rule on organic farmers' pest management techniques was mixed. A number of active ingredients and pesticide formulations that were previously allowed by some—though not all—organic certifiers and state programs were prohibited under the NOP Rule. As a consequence, growers and their suppliers found themselves out of compliance. Although a period of regulatory uncertainty initially existed regarding some

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Table III. Use and Frequency of Disease Management Strategies or Materials (in %) by U.S. Organic Farmers in 1999

Strategy or Material	*Never (%)*	*Rarely or as a Last Resort (%)*	*On Occasion (%)*	*Frequently or Regularly (%)*
Crop rotations	15	1	4	80
Disease resistant varieties	22	3	22	53
Compost or compost tea application	33	7	22	38
Companion planting	42	9	27	22
Sulfur or sulfur-based materials	60	14	14	12
Copper-based materials	66	15	12	7
Solarization	76	10	10	4

NOTE: Based on survey responses of around 1000 to 1100 per category.

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historically approved pesticide formulations, this situation has largely been resolved as products have been re-formulated, the certification agencies have clarified the requirements for product review to operators, and alternative formulations that are compliant have been identified.

Synthetic substances that do not appear on the *National List* are prohibited. Until petitioned substances complete the regulatory process from its initiation as a petition, through the NOSB review, and finally through formal rulemaking, they will not be placed on the *National List* and are thus not allowed in organic production. While this can be a lengthy procedure, there have been two amendments to the *National List* since initial publication in 2000. A successful petition was made for addition of certain inert ingredients. As the result of this petition, the *National List* was amended to permit any inert considered to be of unknown toxicity (EPA List 3) for use in passive dispenser type of pheromone applications. The NOSB also reviewed the generic material spinosad in 2002, and determined that as a natural substance it may be used without appearing on the *National List.*

The NOP has benefited by interaction with EPA to facilitate compliance with the NOP Rule. As would be expected with any new regulatory program, the relationship between the agencies is relatively undeveloped. EPA reclassified over 30 inert ingredients contained in formulations used by organic farmers from List 3 (unknown risk) to List 4 (minimum risk) status before the October 2002 implementation date of the NOP Rule, and as part of the mandated tolerance reassessment under the Food Quality Protection Act. More NOP-compliant pesticides are registered now than were registered at implementation. EPA officials have made a number of presentations to the NOSB and have publicly indicated that they are supportive of the NOP.

Expected Impacts

With the establishment of the organic program at USDA and recognition by EPA, formulators and registrants are able to develop and launch products specifically targeted for the organic market in a way that they were reluctant to do prior to the establishment of the NOP Rule because they are now given clear standards. Formulators have provided data to meet EPA's data requirements, reformulated their products to comply with the NOP Rule, or have petitioned for specific review of their inert ingredients. Farmers have adapted their pest management strategies to take into account the NOP Rule. Above all, consumers expect pesticide products used in organic production to be minimum risk and least toxic formulations. Given that the NOP Rule meets or exceeds other standards in many ways and has been a driving force for innovation in organic farming in other ways, the EU and other standards are likely to be revised in ways that will harmonize with the U.S.

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Formulating Products for the Organic Market

To formulate pesticide products for the organic market in the United States, all of the ingredients—whether active or non-active—need to comply with FIFRA and the NOP Rule. FIFRA is based on the registration of pesticides for efficacy, safety, and environmental protection. Registrants are required to collect and submit data, and have their formulations and labels reviewed and approved by EPA. Each pesticide product is assigned a registration number. A given pesticide product has at least one basic formulation, but a registrant may choose to submit a number of alternate formulations that have the same active ingredient in the same guaranteed percentage, but may have different combinations of non-active ingredients. OFPA recognized FIFRA's primacy and organic farmers are required to obey all pesticide laws under FIFRA as well as the NOP [7 USC 6519(f)]. Additions to the *National List* are made based on the petition process and consideration of criteria related to the impact of the petitioned substance on human health and the environment, availability of alternative methods or materials, and compatibility with organic production systems.

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Active Ingredients

Most non-synthetic (natural) substances are allowed with a few exceptions and most synthetic substances are prohibited for use in organic production. Non-synthetic substances that are not prohibited on the *National List* may be used as both active and non-active substances. Synthetic active ingredients must appear on the *National List* subject to restrictions on use (e.g. insect, disease, weed) and often limitations on application (e.g. specific crop or target pest).

Non-synthetic pesticide products used in organic production include biologicals such as *Bacillus thuringiensis,* botanicals like neem and pyrethrum, and mined minerals such as diatomaceous earth and kaolin clay. A list of selected allowed natural substances used as pesticides—both registered and exempt from registration—appears in Table I. The *National List* prohibits a few natural substances based on adverse effects on human health and the environment. Among the natural pesticides that appear on the prohibited natural list are arsenic, lead, nicotine, sodium fluoaluminate (cryolite), tobacco, and strychnine.

Some synthetic substances have been added to the *National List* for specific uses and applications. In a number of cases, the active ingredient is not intended to be in direct contact with the crop or soil. For example, ammonium carbonate traps can be used only as bait in traps and cannot be directly applied to crop or soil. Boric acid used for structural pest control must be applied in a way that avoids contact with organic food or crops. Copper products, such as copper sulfate, copper hydroxide, and copper oxide are allowed for disease, algae, and shrimp control with a number of restrictions designed to minimize the accumulation of copper in the soil. The antibiotics streptomycin and oxytetracycline as well as peracetic acid are allowed only for the control of fireblight. Sulfur smoke bombs for rodent control are restricted to underground use.

Non-Active Ingredients

Under the NOP Rule, all of the inert ingredients contained in a product must be either natural or, if synthetic, must be classified as minimum risk (List 4) by EPA. Inerts of unknown toxicity (List 3) may be petitioned and considered on a case-by-case basis. An exception to allow List 3 inerts was made for pheromones in passive dispensers. Formulators should first look at the list of minimum risk inert ingredients when selecting inert ingredients. A number of surfactants, spreader-stickers, and solvents used in formulations of biologicals, botanicals, and allowed synthetics still have data gaps and have not been classified as List 4. EPA is in the process of review of pesticides, including inert ingredients as required under the Food Quality Protection Act (FQPA), and this assessment is due in August 2006. By that time, all inert ingredients granted exemption from tolerance during this round of review will be also be placed on List 4.

Compliance Verification

The USDA accredits a variety of public and private entities to certify organic producers and handlers, who in turn may sell products using the USDA seal. The certifier is responsible for verifying that products used by farmers meet the requirements of the *National List*. Certifiers must review both the active and non-active ingredients of pesticide products for compliance. Nonagricultural products used as inputs in organic agriculture cannot carry the USDA organic seal or be considered "certified organic."

Many certifiers use the services of the Organic Materials Review Institute (OMRI), a non-profit established to provide this service of product review. Those agencies that use OMRI services also often review additional products as well. Some agencies conduct their own product reviews, but in all cases a certified farmer must be sure that any products used on the farm are approved by his/her certification agency for use in organic production. Use of a prohibited material on an organic farm could result in loss of certification for 36 months.

Those who apply pesticides in organic production must also comply with restrictions that limit their use. Producers who use botanicals, biologicals, and synthetic substances that are on the *National List* must be justified in the Farm Plan [7 CFR 205.206(e)]. Certifiers use the Farm Plan as a contract to show that the farm meets NOP standards. Because an entire formulation needs to comply with the standard, certifiers and inspectors need to know the brand names used by the farmer.

The EPA has developed a voluntary labeling program that permits a label claim stating "for organic production" for pesticides meeting guidelines compliant with the NOP Rule for no extra fee for registrants. The EPA provided guidelines for pesticide registrants to label pesticide products that meet NOP standards. (*12*). This service provides growers with an additional mechanism for assurance that the pesticide meets NOP requirements for organic production.

All basic and alternate formulations assigned a given EPA registration number must comply with NOP Rule in order for any of the products to make an organic claim. In every case, the non-active ingredients must comply as well as the active ingredients. Formulators who wish to maintain different formulations that contain certain prohibited active or inert ingredients (non List 4) must register the NOP-compliant products under a different registration number than the non-compliant formulation, if they wish to use the EPA organic label.

If a product bears multiple label uses, some of which are within the *National List* restrictions and others not, the product cannot be labeled with an

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organic statement. For instance, boric acid may only be used as an active ingredient for structural pest control with no contact on crops. NOP compliant products are not required to bear an EPA "for organic production" label (Figure 1) in order to be eligible for use in organic systems. A label can make other organic claims as long as they can be substantiated and are not false or misleading, and are approved by EPA in the registration process.

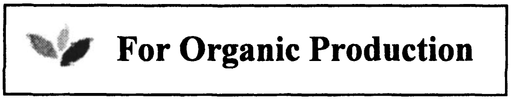

Figure 1. EPA permitted language and logo

Products that are considered minimum risk pesticides that are exempt from EPA registration under section 25(b) of FIFRA are not eligible for EPA review of organic labeling since these products are not registered with EPA. Such products may claim to meet requirements of the NOP, however the formulators are subject to enforcement action under FIFRA for mislabeling if any claims are inaccurate.

Research and Development Opportunities

The expansion of organic agriculture presents opportunities for researchers and product developers to serve the needs of this market. Public investment in organic research projects within the USDA and land grant universities has disproportionately underfunded organic research relative to its market share and growth. The Organic Farming Research Foundation (OFRF) found that less than one-tenth of one percent of USDA's research portfolio had a strong organic focus (*13*). Since that finding, some progress has been made, but organic production does not receive public resources proportional to its share of agricultural sales or resources, particularly when the rapid growth of the organic sector is taken into account (*14*).

Because organic farms depend on making a complex agroecosystem through increased diversity of crops, habitat management, and biological activity, outcomes such as yield, pest pressure, and environmental impacts are determined by the interrelationships among environmental conditions, management, and biological processes (*15*). Factors that limit production seldom can be isolated to a single cause, and rarely have a single easily controlled solution. Organic farming research is best conducted through a multidisciplinary and holistic systems approach that involves farmers in the research process and

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incorporates on-farm experimentation to complement work conducted on research stations (*16*).

Organic farmers have special resistance management needs, given that they have a limited number of pesticides that they can use in rotation for any given target pest. The introduction of transgenic crops may accelerate resistance to Bt and thus increase the urgency to develop alternatives for organic farmers. Various Lepidoptera such as diamondback moth *(Plutella xylostella)* (*17*) and Coleoptera such as the Colorado potato beetle (*Leptinotarsa decimlineata)* are known to have strains resistant to *Bt* (*18*). Research on resistance management strategies compatible with organic standards will require special attention given the limited number of pesticides that are available. Researchers and formulators will need to consider alternative methods and strategies to control pests in a way that maintains susceptibility for resistance management strategies to be effective in an organic farming system.

Additional research and development is needed to improve the efficacy of formulations that contain only minimum risk inerts. One possible approach is to substitute List 4 inerts for those that are not on List 4, run efficacy trials, and optimize the formulation. The other is to add more inert ingredients to List 4. Data gaps on various substances—particularly solvents for botanicals—need to be closed in order for them to be eligible to be classified as minimum risk inerts. Research is needed to help identify what substances of unknown toxicity are the most promising candidates for reclassification as minimum risk. Once these substances are identified, then the studies need to be conducted and the data submitted to EPA so that they can be reclassified.

Pheromone disruption has helped to reduce costs, increase yields, and improve the quality of organic apples by managing the levels of codling moth (*Cydia pomonella)* (*19,20*). Passive dispensers are permitted to use inerts of unknown toxicity as well as minimum risk inerts, but other delivery systems have not. Other chemicals used in communication (semiochemicals) have similar potential if systems can be developed to deliver them efficiently with minimum risk inerts. One specific need is the development of propellants that are classified as minimum risk so that pheromones can be consistently dispersed over a wide area for an entire season without harming the environment or threatening human health.

Natural, non-toxic alternatives to copper and sulfur for the management of various fungal and bacterial diseases also offers a promising need. Organic farmers in the U.S. already face restrictions on the use of copper to prevent accumulation in the soil. The EU has established restrictions on the use of copper that are scheduled to increase over time (EC 2092/91 as amended by EC 473/2002). Microbiological antagonists and other forms of biological control may be viable for certain specific diseases. Inconsistent data on efficacy of microbial controls such as *Bacillus subtilis* and *Beauveria bassiana* merit further

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trials (21). Clay barriers, similar to kaolinite, also may serve as substitutes for copper and sulfur fungicides.

While most organic farmers do not use botanicals, there are situations where crop production costs can be lowered and quality improved by the use of a natural insecticide. Research in least toxic knock-down insecticides and various insect repellants may offer better long-term solutions for organic farmers than the use of broad-spectrum biocides. The development of products that are exempt from EPA registration may be more promising and expedient, but real breakthrough products will likely require experimental use permits and full registration.

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Conclusions

Organic agriculture offers an exciting opportunity to reduce pesticide risk, meet consumer expectations, and supply a growing market. Organic standards require farmers to practice methods that are less harmful to the environment and take into account considerations of public health. Organic techniques can be further improved through the development of biologically based and systems oriented solutions to crop protection challenges.

Information Resources

Appropriate Technology Transfer for Rural Areas (ATTRA) is funded by the US Department of Agriculture, is a national sustainable agriculture information service managed by the National Center for Appropriate Technology. It provides information and other technical assistance to farmers, ranchers, Extension agents, educators, and others involved in sustainable agriculture in the United States. ATTRA maintains publications on their website that were written largely in response to questions from farmers. ATTRA publications on organic farming include guides for most widely produced organic crops. URL http://attra.ncat.org/

The **Bio-integral Resource Center (BIRC)** publishes the *IPM Practitioner* and *Common Sense Pest Control Quarterly*. BIRC serves as a resource center for Integrated Pest Management (IPM) and least toxic pest control methods. While their publications are not limited to giving information that complies with organic standards, much of their information is useful for organic producers and handlers. URL http://www.birc.org/

Because the **EPA** is responsible for regulating pesticides, it is an information source in the development of new pest management tools for organic farmers. EPA is also the official source for information on risk classification of inert ingredients (List 1-4). URL http://www.epa.gov/opprd001/inerts/lists.html

The official records of the **National Organic Program** are contained on a website maintained by the USDA. The website contains the NOP Rule and the process by which it was made. News on petitioned substances, TAP reviews, announcements of NOSB meetings, and other proposed revisions and actions also appear on the website. URL http://www.ams.usda.gov/nop

The **Organic Farming Research Foundation (OFRF)** is a non-profit whose mission is to sponsor research related to organic farming practices, to disseminate research results to organic farmers and to growers interested in adopting organic production systems, and to educate the public and decision-makers about organic farming issues. URL http://www.ofrf.org

The **Organic Materials Review Institute (OMRI)** publishes a *Generic Materials List* that is a reference guide than enables producers, handlers, certifiers, formulators, and suppliers to quickly look up whether or not a given input or ingredient complies with the NOP Rule (*22*). OMRI also publishes a *Brand Name Products List* of proprietary products that are allowed for use under the NOP Rule. OMRI's *Operating Manual* explains the procedure for the evaluation of the products that appear on the *Brand Name Products List.* URL http://www.omri.org

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Chapter 3

Organic Certification of Pesticides: From Philosophy to Practice

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Organic agriculture, like all human activities, is practiced in a way that is consistent with a philosophical point of view. Scientific data are used to determine if materials, including those with pesticidal properties, are consistent with a belief system that places a high value on biodiversity and the enhancement of biological cycles and soil biological activity. How the USDA National Organic Program incorporates these beliefs is discussed.

Introduction

Organic agriculture begins with a philosophical point of view. This is not unique to organic agriculture or even to agriculture in general but is a component of all human activities. Traditional agriculture, as practiced since the late 1950s and early 1960s, begins with a goal of maximization of production to feed the existing and future human population. When humans arrived at a point where we could reduce the impact of droughts and other natural disasters on food supplies, we were able to implement the philosophical viewpoint that we are ethically required to do everything we can to feed all people. This

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
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philosophic viewpoint had minimal concerns about the impact of agriculture on surrounding ecosystems. We irrigated without considering or understanding the impacts of moving water from one location to another, we added fertilizer without considering the impact of these nutrients on surrounding ecosystems, we applied pesticides without considering the impact on beneficial organisms, and we increased our reliance on fossil fuels without considering the impacts from releasing large quantities of carbon into the atmosphere. While maximizing production to feed the human population is still the focus, the philosophic viewpoint of sustainable agriculture requires concern about the impact of agricultural practices on surrounding ecosystems. Because of the agroecosystem's connection to and dependence upon surrounding ecosystems, this philosophical viewpoint does not support the belief that it is possible to feed the human population without being aware of and working with the ecosystems surrounding the agroecosystem. Organic agriculture in the United States, like sustainable agriculture, supports the belief that all ecosystems, whether managed or unmanaged by humans, are interdependent but this philosophical viewpoint does not accept that one can honor the interdependency while using synthetic materials. Organic agriculture in the United States differs from organic agriculture in some other countries in that the focus is on the processes used to produce our food rather than testing of the product for unacceptable materials prior to consumer purchase. Again, this difference is a result of a philosophical difference. The former emphasizes the interdependence of systems and organisms while the later emphasizes the impact of synthetic material residues in food on humans.

Organic certification of pesticides is considered by many within the organic community to be inconsistent with organic agriculture and somewhat oxymoronic. Philosophically, proponents of mainstream organic agriculture, sustainable agriculture or IPM do not accept that any chemical should be used, unless all other methods for addressing an issue have been tried first. If all other methods fail, then the least toxic material may be used with caution. The list of materials available to the organic producer is the most restricted. A pesticide is viewed as a symptom of a larger problem – the interactions between various organisms in the agroecosystem are not in balance. The focus of a producer's or handler's efforts should be to restore the balance rather than to use a material that will impact non-target organisms and not provide a long-term solution for the problem.

The views of agriculture described above are an oversimplification of each philosophical viewpoint (worldview). Those who describe themselves as adherents of traditional, sustainable or organic agriculture will not necessarily agree with the above description nor will they necessarily agree with the description of others who state that they practice traditional, sustainable or organic agriculture.

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The National Organic Program[1] (NOP) is a result of an amalgamation of various worldviews and science. The worldviews that merged come from a number of alternative communities including those focused on concepts such as the natural foods, small-is-beautiful, back-to-the-land, and environment. These communities were able to agree on food production practices, but the concerns that created the desire to produce food using organic agricultural methods, the reasoning for particular practices, and views on how to achieve goals differs among the communities. How the Organic Food Production Act (OFPA), enacted by the United States Congress in 1990, intersects with other goals of each community also differ, thus creating tensions and disagreements among producers, advocates, and consumers of organic foods.

One item the various communities agreed was important was the use of science for decision-making. While everyone agreed science should be the basis for decisions, it was recognized that science is practiced within a cultural context and conclusions reached by one person may not be the same as conclusions reached by another person. This will especially be true when worldviews differ. This is the major reason why some individuals will believe that a particular decision by the USDA National Organic Standards Board (NOSB) was science-based while others will believe that non-scientific opinion drove the decision.

The influence of worldview on science is obvious within the NOP because the philosophical viewpoints within the organic community are not in the mainstream and are varied. When the views become mainstream the use of opinion for decision making rather than science will appear to occur less often because fewer people will disagree with the assumptions that underlie the interpretation of the scientific data.

What is the National Organic Program?

As a food labeling law, the OFPA placed the administration of the National Organic Program within the Agricultural Marketing Service of the United States Department of Agriculture. It is very important to note that OFPA addresses organic food production, not the production of non-food items. It is also important to understand that OFPA regulates how food is produced and processed, not what is in food. Thus, it does not guarantee that pesticides will not ever be found in food labeled organic. Food that is labeled organic will not have been produced with any synthetic material, unless the material has been approved for addition to the National List by the NOSB and added to the List by the United States Secretary of Agriculture. OFPA also allows for the prohibition of natural (non-synthetic) materials.

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[1] In this paper, the National Organic Program refers to organic farmers, processors, retailers, and consumers, the National Organic Standards Board, the National List, the Rule, and all aspects of the organic agricultural program that were created by the Organic Food Production Act. It does not refer to the specific people or actions of the USDA National Organic Program office.

An additional important characteristic of OFPA is its consumer rather than producer/processor focus. Many supporters of OFPA are less interested in creating conditions under which all types of food may be labeled as "organic" than providing food that has been produced and processed under conditions that are believed by supporters to be healthy and environmentally benign. A significant segment of the organic community believes that not all foods can be labeled "organic". If soil conditions require the addition of macronutrients, then food grown in this location cannot be labeled "organic". If an animal requires the administration of antibiotics to maintain health, the antibiotic must be administered but the animal cannot be labeled "organic". Thus a tension is created between producers/processors and consumers. A producer or processor may advocate the addition of synthetic materials to the National List because the materials are essential for production or processing whereas a consumer may not wish for a synthetic material to be added even if it means that the raw or processed food cannot be labeled "organic". OFPA favors the consumer viewpoint.

The motivation of those who grow, process, sell and buy organic foods (the organic community) differs tremendously. Reasons vary from perceived environmental and health considerations or social benefits to economic gain. For many years the organic community argued about the usefulness and constraints posed by a national standard. Arguments against a national standard included greater emphasis on local control, flexibility, and a distrust of the federal government, whereas arguments in favor included expansion of the organic market, product comparability and consistency, leveling of the playing field for producers and retailers, and elimination of questionable practices within an unregulated community.

The passage of OFPA by Congress was the culmination of discussions beginning over a decade earlier concerning the positive and negative attributes of having a single set of rules governing the use of the word "organic". The organic agricultural community had concluded by the late 1980s that a set of uniform standards administered at the Federal level, under which production and processing of food would occur, would be advantageous to the industry. The law created a short-cut for consumers such that it would no longer be necessary to investigate every item on a food label or to know the standards used by the myriad of organic food certifiers. OFPA also increased the likelihood that organic foods would be found in supermarkets because marketing would be easier.

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What Does Organic Mean in the Context of OFPA?

In 1990, Congress created a legal definition of the word "organic" and context for its use. Organic according to OFPA is "a labeling term that refers to an agricultural product produced in accordance with the Act and the regulations in this part" (*1*). Within the context of the law, the agricultural production will be carried out in a sustainable manner, comply with the Final Rule (*2*), and be limited in the types of pesticides used in production to non-synthetic materials that are not prohibited or synthetic materials that are allowed.

To use the USDA Organic label, a producer or handler must have an organic production and handling system plan and be certified by a USDA accredited certifier. The production and handling system plan must include "(1) a description of the practices and procedures to be performed and maintained, including the frequency with which they will be performed; (2) a list of each substance to be used as a production or handling input, indicating its composition, source, locations(s) where it will be used, and documentation of commercial availability, as applicable; (3) a description of the monitoring practices and procedures to be performed and maintained, including the frequency with which they will be performed, to verify that the plan is effectively implemented; (4) a description of the recordkeeping system implemented…; and (5) a description of the management practices and physical barriers established to prevent commingling of organic and nonorganic products on a split operation and to prevent contact of organic production and handling operations and products with prohibited substances" (*3*). In addition to following the written organic production and handling system plan, a producer or handler must follow applicable regulations in the Final Rule concerning land, soil fertility, and crop nutrient management; seed and planting stock; crop rotation; crop pest, weed, and disease management; wild-crop harvesting; livestock health care, origin, feed and living conditions; and food handling, including facility pest management practices. While prevention of pests and the use of natural processes for soil fertility or food process are preferred, synthetic chemicals may be used if they are on the National List.

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The National List

Organic production, as defined by OFPA, is limited to non-synthetic materials that are not prohibited and allowed synthetic materials. Prohibited non-synthetic substances and allowed synthetic substances are contained in the National List (See Tables I - III for examples.) For a material to be considered for addition to or deletion from the National List, a petition must be submitted to the USDA National Organic Program office and considered by the National Organic Standards Board (NOSB). Only the NOSB can recommend materials for addition or deletion from the National List. When considering a material, the NOSB may only evaluate single substances or ingredients; no formulated products can be included on the List. If the NOSB recommends the addition of a synthetic material or the removal of a non-synthetic material, then the substance is added to or deleted from the List by the Secretary of Agriculture. The material cannot be used until it is on the National List. Materials on the National List are reviewed every five years.

Additionally, the NOSB may be asked to determine if a material is non-synthetic, thus eliminating the need to add the material to the National List. This situation typically occurs when a divergence of opinions exists about the synthetic or non-synthetic status of a material. Spinosad is an example of this situation. Some individuals believe spinosad is a non-synthetic while others believe it is too highly processed and thus a synthetic material. To resolve the situation, spinosad was petitioned for addition to the National List as an allowed synthetic material. The NOSB determined at its May 2002 meeting that spinosad is a non-synthetic material and, thus, allowed without being added to the National List. Other materials, e.g., soy protein isolate, are currently awaiting a decision on their synthetic or non-synthetic nature.

Currently, the National List is divided into three major categories: Crops, Livestock and Handling. It is necessary for a substance to be listed in the category for which it will be used. For example, if a synthetic material is to be used on crops, it must be listed within the crops category. These categories are not part of the original legislation and can be changed depending upon the perceived usefulness of this or other means of organizing the information.

How Are National List Decisions Made?

The review of a material begins with the submission of a petition. Anyone may submit a petition to the NOSB seeking the evaluation of a substance for inclusion or deletion from the National List. Five types of actions can be petitioned: 1) addition of a synthetic substance for use in organic crop or livestock production, 2) removal of a synthetic substance, 3) prohibition of a non-synthetic substance in organic crop or livestock production, 4) removal of a non-synthetic substance from the prohibited list, and 5) addition of a nonagricultural (nonorganic) substance allowed in or on process products labeled as "organic" or "made with organic (ingredients are specified on label)."

When a material is petitioned for addition to the List the petitioner submits information pertaining to the substance to the NOSB. The information submitted includes a) substance common name and Chemical Abstract Service (CAS) number; b) manufacturer's name, address and phone number; c) intended or current use of the substance; d) a list of desired crop or livestock uses along with application method and rate or a list of processing activities for which the

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Table I. Selected Materials on the National List - Crops

SYNTHETIC ALLOWED	*NON-SYNTHETIC PROHIBITED*
Disinfectants	Arsenic
Ethanol	Lead salts
Hydrogen peroxide	Sodium fluoaluminate
Insecticides	Strychnine
Boric acid	Tobacco dust (nicotine sulfate)
Lime sulfur	Potassium chloride
Plant disease	Sodium nitrate
Fixed copper	
Lime sulfur	
Soil amendment	
Humic acid	
Soluble boron	
List 4 inerts	

Table II. Selected Materials on the National List - Livestock

SYNTHETIC ALLOWED	*NON-SYNTHETIC PROHIBITED*
Disinfectants	Strychnine
Ethanol	
Electrolytes	
Hydrogen peroxide	
Sodium hypochlorite	
Topical Treatments	
Iodine	
Copper sulfate	
Mineral oil	
Feed Additives	
Vitamins	
Trace Minerals	
List 4 inerts	

substance will be used plus its mode of action; e) source(s) of substance and description of how substance is manufactured (if confidential business information (CBI) is involved, then information can be submitted to justify the deletion of CBI from the public portion of the petition); f) a summary of previous reviews by State or private certification programs; g) information on EPA, FDA and/or State regulations, including registration numbers; h) labels of products containing the substance; i) physical properties and chemical mode of

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Table III. Selected Materials on the National List - Handling

SYNTHETIC ALLOWED	*NON-SYNTHETIC ALLOWED*
Ascorbic acid	Alginic acid
Calcium citrate	Citric acid
Carbon dioxide	Bentonite
Ethylene	Calcium carbonate
Ferrous sulfate	Dairy cultures
Glycerin	Kaolin
Hydrogen peroxide	Potassium chloride
Bleached lecithin	Sodium carbonate
Pectin	Carnauba wax
Potassium acid tartrate	Wood resin
Potassium citrate	Bakers yeast
Sodium citrate	Brewers yeast
Xanthan gum	Nutritional yeast

action including interactions with other substances; j) toxicity, effects on human health, including safety information from a MSDS, environmental persistence, and effects on soils, livestock and/or crops; k) environmental impacts from manufacturing, use, misuse or disposal; l) comprehensive review of the research on the substance, including a bibliography; and m) a petition justification statement.

The petitioner information provides the basis for a Technical Advisory Panel (TAP) review. A TAP report contains all the information submitted by the petitioner, the current and historical use in the organic industry both nationally and internationally, an evaluation of possible detrimental chemical interactions with other materials used in organic farming systems, an assessment of the potential for environmental contamination during manufacture, use, misuse or disposal, a description of potential alternatives in terms of practices or other available materials, and an evaluation of compatibility with a system of sustainable agriculture. The report is then reviewed by at least 3 technical experts. The report and reviews are submitted to the National Organic Standards Board.

The National Organic Standards Board, using the TAP report and the reviews written by the technical experts, evaluates the material based upon criteria provided within OFPA. The first decision required by OFPA is a determination of the synthetic or non-synthetic nature of the material. If a material is determined to be synthetic and is being petitioned to be added or removed from the National List or is non-synthetic and being petitioned to be prohibited, three criteria are used in the evaluation: 1) adverse impacts on humans or the environment; 2) need for the material in organic production; and

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3) compatibility of the substance with organic production practices. If a material is determined to be non-synthetic, no further action is required of the NOSB.

Adverse Impacts on Humans or the Environment

When evaluating the potential for adverse impacts on humans or the environment the Board is required to consider adverse effects from manufacture, use or disposal, adverse interactions with biological, chemical or other materials used in the agroecosystem, physiological effects on soil organisms, livestock or crops, and undesirable persistence or metabolites. If the material is used in the handling of foods after production, additional data need to be included in the evaluation. The potential for adverse effects on human health from exposure to the material must be considered. The status of the material as GRAS (Generally Recognized as Safe) when used according to good manufacturing practices, as determined by FDA, will be included in the assessment along with the potential for residues of heavy metals or other contaminants.

Need for the Material in Organic Production

The need for the material in organic production is determined using information on the availability of alternative substances or practices, natural sources, and wholly natural or organic substitutes. Non-synthetic materials are preferred over synthetic materials even if both may be used interchangeably.

Compatibility of Material with Organic Production Practices

The compatibility of a material with organic production includes an evaluation of the expectation of the organic consumer regarding the authenticity and integrity of organic products. An evaluation is conducted of the consistency of the material with the principles of sustainable agriculture, such as the long-term viability of organic farm operations, the encouragement of preventative techniques including cultural and biological methods for management of crop, livestock, and/or handling operations and the use of renewable resources. OFPA states that preservatives are not consistent with organic agriculture and are not allowed. Additionally, the re-creation or improvement of flavors, colors, textures or nutritive values lost in processing is not allowed.

According to OFPA, to include a synthetic substance on the National List it must contain copper or sulfur compounds or be a toxin derived from bacteria, a pheromone, soap, horticultural oil, fish emulsion, a treated seed, vitamin, mineral, or livestock parasiticide or medicine or a production aid such as netting,

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tree wraps, insect trap, sticky barrier, or equipment cleaner. Other synthetic materials are by definition not allowed to be added to the National List.

Case Study: Evaluation of Ozone by the National Organic Standards Board

Ozone is an example of a synthetic material that was petitioned for use. The petitioner stated that the intended use was as an antimicrobial agent in irrigation lines and for weed control. The petitioner provided information on the rate and method of application, the manufacturing process, physical properties (strong oxidizing agent), mode of action (lyses membranes), and safety, along with other data considered by the petitioner to be important for successful review of the material by the NOSB. The Technical Advisory Panel report incorporated information from the petitioner with information on the historical use of ozone, its FDA status (GRAS as a direct food additive and as an antimicrobial agent for bottled water and food processing), its potential to interact with soil organisms if used as a herbicide, human health effects, lack of persistence, and other relevant information. The technical expert review produced mixed results. One reviewer concluded that the data did not support the use of ozone in organic agriculture while the other two reviewers concluded that ozone could be used with restrictions. The NOSB concluded that weed control was not an acceptable use of ozone within an organic system because of ozone's adverse impact on soil organisms and the availability of other weed control methods. Ozone as an antimicrobial agent in irrigation lines was determined by the NOSB to be acceptable because the method of application and quantity of material used limits the potential for adverse human or ecosystem impacts and alternative methods for cleaning irrigation lines are limited.

Conclusion

Organic agriculture began with a philosophical point of view, as do all components of all human activity. With the passage of OFPA in 1990 organic agriculture moved from a multitude of standards, determined with the use of scientific information that had been evaluated by individuals with a narrow range of worldviews, to one uniform standard determined by combining scientific information with formally obtained input from a broad spectrum of the American public.

The National Organic Standards Board has defined organic agriculture as "an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity. It emphasizes the use

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of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. These goals are met, where possible, through the use of cultural, biological, and mechanical methods, as opposed to using synthetic materials to fulfill specific functions within the system" (*4*).

The National Organic Program uses scientific data to evaluate the acceptability of various materials and methods within organic agriculture. All synthetic materials, whether they have pesticidal properties or not, must be consistent with the definition of organic agriculture contained in OFPA and The Rule and be placed on the National List through a recommendation of the National Organic Standards Board. Approved materials, including those with pesticidal properties, only may be used when other methods to control pests have failed.

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2. 7 CFR Chapter I subchapter M part 205, National Organic Program; Final Rule
3. 7 CFR Chapter I subchapter M part 205 Subpart C section 205.201
4. NOSB Principles of Organic Production and Handling, Adopted October 17, 2001, National Organic Standards Board Policy Manual, page 32, adopted October 19, 2002, revised May 14, 2003.

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Chapter 4

The IR-4 Program for Registration and Development of Organic Products and Biopesticides

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This chapter describes the activities of the IR-4 Project with biopesticide products and how IR-4 promotes registration and efficacy research for their adoption and development. It also compares and contrasts biopesticide and organic products as well as discussing specific regulatory issues around labeling organic products.

Background

The term biopesticide probably has as many varied definitions as the term organic. Biopesticides are often thought of as being synonymous with organic or natural. While the term organic is officially defined and pesticidal products are designated by the National Organic Program of the United States Department of Agriculture (USDA) (*1*), biopesticides are officially designated by the Biopesticides and Pollution Prevention Division (BPPD) of the United States Environmental Protection Agency (EPA) (*2*). The Biopesticide and Pollution Prevention Division of EPA was initiated in 1994. Products that are

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

currently considered biopesticides have been registered since the 1940's even though the individual division was not developed until 1994. The first record for products that are currently considered biopesticides is for oil of mustard as a repellent, and the first microbial pesticide registered was *Bacillus popilliae* for control of Japanese beetles. Products with these first biopesticides are still available today. The registration of biopesticides has its own set of requirements that are independent from organic status. An overview of the registration process for biopesticides has been summarized (*3*).

Biopesticides are certain types of pesticides derived from animals, plants, bacteria, and certain minerals (*4*). These are defined by EPA's BPPD into three main classes as follows:

1. **Microbial pesticides** are products consisting of a microorganism such as a bacterium, fungus, virus or protozoan as the active ingredient.
2. **Plant-Incorporated-Protectants (PIPs)** are pesticidal substances that plants produce from genetic material that has been added to the plant using modern biotechnology methods.
3. **Biochemical pesticides** are naturally-occurring substances that control pests by a non-toxic mode of action. Biochemical pesticides include substances that create barriers against pest attack, cause suffocation of insects, and pheromones that interfere with mating by leading male insects to traps or otherwise making it harder to locate females. There are also non-pheromone type attractants or scents that are used to lure, trap, or when combined with toxicants to kill insects. Synthetic analogs of biochemicals can also be registered. One of the more recently developed products induces a plant to invoke its defense systems against pathogens. While microbial and PIP products are well defined, the requirement of a non-toxic mode of action is often the key distinction between a biochemical biopesticide and a conventional pesticide. BPPD has a specific group of scientists called the Biochemical Classification Committee that determines whether a substance meets the criteria for classification as a biochemical pesticide.

Plant incorporated protectants such as those that contain genetic material coding for production of *Bacillus thuringiensis* (Bt) are not considered to be organic under USDA organic rules (*1*) but sprayable forms of Bt are listed by the Organic Materials Review Institute (OMRI). In contrast, both Bt in sprayable forms and derived from PIP are regulated as biopesticides by EPA. There are a number of microbial derived or biochemical products that are allowed under organic rules such as tetracycline but most antibiotics are not biopesticides. Products that have organic designations such as spinosyn and pyrethrum are not considered to be biopesticides by EPA, primarily because of their direct toxic mode of action.

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The IR-4 Project

The IR-4 Project is funded by the USDA Cooperative State Research Education and Extension Service (CSREES) and the Agricultural Research Service (ARS) and also receives support from the directors of state agricultural experiment stations. IR-4 is an applied research program whose mission is to help specialty crop producers obtain safe and effective pest control products. The program was initiated in 1963 and historically has focused on registration and reregistration of chemicals and biopesticides for use on specialty crops or for minor uses on major crops.

IR-4 broadened its scope in 1982 to include research leading to registration of a wide range of biopesticides including microbial biopesticides, nonviable microbials, genetically altered microbials, transgenic plants and biochemicals . The program is committed to developing alternative pest control products on minor food crops and ornamentals by working cooperatively with public and private sector individuals and organizations. IR-4 interacts with the USDA, EPA, and product registrants to determine the requirements for registration of proposed uses. The program has the resources to develop research protocols, assist with Experimental Use Permits, coordinate and fund field and laboratory research, assist in the development of Tier I toxicology and non-target organism waivers, and prepare data packages for submission to the EPA. IR-4 residue research is conducted according to EPA Good Laboratory Practice regulations utilizing the IR-4 Quality Assurance network.

Under the under Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the EPA regulates all materials that claim to have pesticidal properties. In general, the number and type of studies required to register biopesticide products are different from the studies required to register conventional pesticides. IR-4 will consider biopesticides that meet the EPA definition as well as other low exposure, naturally occurring biochemicals which have pest control activity, provided they do not have significant toxicity to man, mammals, fish or birds.

Biologicals such as arthropod (insect) parasites and predators or predacious nematodes are not regulated under FIFRA and because of this do not fall under the purview of the IR-4 program.

IR-4 Grants for Biopesticide Research

The primary objective of the IR-4 Biopesticide Research Program is to further the development and registration of biopesticides for use in pest management systems for specialty crops or for minor uses on major crops. Areas of IR-4 assistance include:

1. Arrange preregistration meeting with EPA and developing approved research protocols;

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2. Assist in complying with EPA Good Laboratory Practice regulations;
3. Fund small and large scale field efficacy trials;
4. Fund magnitude of residue trials, if needed;
5. Assist in obtaining Experimental Use Permits from EPA;
6. Prepare and submit petitions to the EPA to support tolerances (maximum levels of substances allowed in raw agricultural commodities) or tolerance exemptions;
7. Develop data to expand registration to include additional crops and uses;
8. Prepare registration documents for submission to the EPA.

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In 1995, IR-4 decided to provide some additional financial support; thus, a competitive grant program was initiated at $300,000 a year. Since 1995, the IR-4 project has funded approximately $3 million dollars in efficacy research. About 25% of research is on early stage projects and 75% on advanced stage projects. The IR-4 Project defines early stage projects as ones involving products that have no or incomplete toxicology packages and advanced stage projects as ones which generally involve expanding the use of registered products. This usually involves efficacy studies leading to adding new crops , or new pests and sites to the existing label. While IR-4 has funded biopesticide research on both organic and non-organic products, for the purposes of this paper only the organic products are listed in Table I.

For unregistered products IR-4 involves EPA in the review process to increase the likelihood that supported products are capable of making it through the regulatory process. Some state regulatory agencies such as California's Department of Pesticide Regulation require efficacy data for registration purposes. The goal of the IR-4 Biopesticide Research Program is to work with university and USDA researchers to develop the data that would help support registrations and to also work with key university researchers and extension agents who recommend to growers what biopesticides can be used.

In the 13-year period between 1982 and 1994 the IR-4 Biopesticide program emphasized support for regulatory work for registrants and IR-4 support resulted in registration of a substantial number of important biopesticides (5). Products that IR-4 has assisted in submitting registration packages for are listed in Table II. Probably the most important product was and still is several sub-species of *Bacillus thuringiensis* or Bt. IR-4 continues to assist registrants which are primarily comprised of very small businesses and individual scientists with limited regulatory experience. IR-4's involvement has varied widely depending on the particular registration. In most instances, exemptions from the requirement of a tolerance are obtained from EPA. However, in some cases, IR-4 has conducted magnitude of residue trials. In cases of insufficient data for support of full registration, an Experimental Use Permit was submitted and granted. There have been several registrations in which IR-4 has prepared and submitted entire data waiver justifications, petitions and registration documents to the EPA.

Table I. Listing of OMRI[a] Allowed and Regulated BiopesticidesSupported by IR-4 Efficacy Research[b]

Active Ingredients	*Trade Name[c]*	*Biotype[d]*
Bacillus pumilus	Yield shield	Fungicide
Bacillus subtilis	Serenade	Fungicide
Candida oloephila	Aspire	Fungicide
Coniothyrium minitans	Contans, Intercept	Fungicide
Gliocladium virens	SoilGard	Fungicide
Hydrogen peroxide	Stor OX	Fungicide
Pantoea agglomerans	Bloom Time Biological	Fungicide
Potassium bicarbonate	Kaligreen	Fungicide
Psudomonas syringae ESC-10	Bio-Save 10 LP	Fungicide
Rosemary oil	Sporan	Fungicide
Streptomyces griseovirdis	Mycostop	Fungicide
Trichoderma harzianum	Plant Shield , RootShield	Fungicide
Azadirachtin and neem	Aza-Direct, Neemix, Trilogy	Insecticide
Bacillus thuringiensis	Numerous	Insecticide
Beauveria bassiana strain GHA	Mycotrol O	Insecticide
Capsaicin	Millers Hot Sauce	Insecticide
Codling Moth Granulosis Virus	Cyd - X	Insecticide
Garlic	BioRepel	Insecticide
Helicoverpa zea + virus	GemStar	Insecticide
kaolin	Surround	Insecticide
Quillaja	Quillaja	Nematicide
aminoethoxyvinyglycine (AVG)	ReTain	Plant Growth Regulator
gibberellic acid	Falgro	Plant Growth Regulator
pheromones	Checkmate OFM	Pheromone

[a] Organic Materials Review Institute; Box 11558; Eugene, OR 97440

[b] Research funding was provided to public research institutions, not OMRI.

[c] Trade names are included only as a reference. The actual product(s) evaluated may or may not be that particular commercial product

[d] Listing of research in this manner does not constitute a recommendation for use. Consult the current label for proper use directions.

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Table II. Registration Clearances Obtained by IR-4 on Behalf of Registrants

Product	*Crop or Use*
Aspergillus flavus AF 36	Cotton-AZ and TX
Bacillus popilliae	Pastures
Bacillus thuringiensis (Bt)	All crops (Label Expansions)
Cinnamaldehyde	Mushrooms (*Verticillium* spot and dry bubble disease control) and for insect and disease control on 53 crops
Codling Moth Granulosis Virus	Apple, Pear, Walnut & Plum
Formic Acid	For mite control in bee hives
Gibberellins	For PGR use on minor crops
Grape Berry Moth Pheromone	Grape
Kaolin	Insect control on 48 crops
Lagenidium giganteum	Rice
Lysophosphatidylethanolamine (LPE)	97 and 98S formulations on 13 fruit crops (EUP) promote ripening and extend storage shelf life.
Methyl Anthranilate	Bird repellent on blueberries, grapes and cherries
Milsana	For powdery mildew control on ornamentals
Sucrose Octanoate	On all commodities for insect and mite control (pending)
Thymol	Section 18 for varroa mite control in beehives
Verticillium WCS850 (Dutch Trig)	Experimental Use Permit for control of Dutch Elm disease
Yeast Hydrolysate	Control of greasy spot on citrus and bacterial leaf spot in tomato

EPA Label Approval under the National Organic Program

The USDA NOP has the authority to list products they designate as organic, but the pesticidal products labeling still falls under the jurisdiction of EPA. This section is drawn from Pesticide Registration (PR) Notice 2003-1 (*6*). PR Notice 2003-1 describes how registrants can obtain EPA approval of label language indicating that all ingredients (active and inert) in a pesticide product and all uses of that pesticide meet the criteria defined in the USDA's National Organic Program (NOP) Rule. Registrants of pesticide products are not required to specify on their label that their products can be used in organic agriculture. However, if a registrant does want to include such language, it must comply with PR Notice 2003-1. Since the USDA already has a system in place to define organic pesticides, the EPA depends on the USDA's listing for deciding if a product qualifies for organic use language to be included on its label.

Except for exempted products (also known as 25b products) the labeling requirements of registered products fall under the jurisdiction of the EPA as established under FIFRA. Among the requirements enforced by EPA is that the labeling not be false or misleading in any manner. There have been perception problems within the agricultural community as well as the general public over the exact meaning of the term 'organic'. EPA has previously regarded statements such as "organic" to be forms of false or misleading safety claims. After creation of the Federal Organic Food Production Act (7 U.S.C. section 6501 et seq.) and associated NOP Rule, consistent Federal standards for what "organic" means now exist. PR 2003-1 has the phrase "for organic production" as suggested label wording to designate organic products because it only states the ability to use the product within organic agriculture without implying any safety claims.

In order for a product to meet the requirements of the NOP, each ingredient in the product, including active and inert ingredients, must be allowable under The National List of Allowed and Prohibited Substances (National List) contained in 7 CFR part 205. All uses on the product label must be eligible or the product should not be labeled with a NOP statement.

The National List of acceptable products for organic agriculture is available at: http://www.ams.usda.gov/nop/NationalList/FinalRule.html. Inert ingredients (including List 4) (specifically allowable for use in crop and livestock production in the National List) are available at: http://www.epa.gov/opprd001/inerts/inerts_list4.pdf.

A tolerance is the maximum allowable concentration of a substance in or on a crop. All ingredients of pesticide products registered (or exempted from regulation under FIFRA) for use on foods must have either a tolerance or an exemption from the requirement of a tolerance if residues of such ingredients result in or on the food from such use.

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The labels for “minimal risk pesticides”, also known as 25(b) or exempted products are not reviewed by the EPA, so EPA cannot approve the use of NOP language on 25(b) product labels. Products exempted under FIFRA section 25(b) are not precluded from identifying whether they meet the requirements of the National Organic Program. However, producers of such products are reminded that it is their responsibility that they meet all other EPA requirements and NOP requirements.

Although there are various types of wording that are acceptable and there are methods for getting new wording approved by the EPA, the phrase mentioned by the EPA is “For Organic Production”. There are methods for utilizing different words and logos, but some phrases in the NOP listing such as “minimizes accumulation in the soil” or statements that are not quantifiable are generally not acceptable. The phrase should be located on the front panel of the label in close proximity to the product name. The phrase should not appear above the product name (in the location normally reserved for a Restricted Use Statement). The font size should be comparable to that of other type and not highlighted by size, color, contrast or placement.

Inclusion of an ingredient on the National List is independent of approval of ingredients for use in pesticide products under FIFRA or Federal Food, Drug and Cosmetic Act (FFDCA). Some of the items on the National Organic list are quite vague such as “dairy cultures’ and “chlorine materials”. Such sweeping inclusions are not approved by BPPD although some specific products may be registered. The National List may include substances that are not currently found in any registered pesticide product; therefore, it is the responsibility of the registrant to ensure that all ingredients are currently approved by the EPA. It is also important that registrants keep abreast of any removal of products from the NOP list or tolerance or registration changes that affect its eligibility for the proper label language.

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Profiles of Selected Biopesticide Registrations Facilitated by IR-4

Aspergillus flavus AF36

Aflatoxin is a known carcinogen produced by *Aspergillus flavus* and other *Aspergillus* species. Aspergillus flavus AF36 is a naturally occurring strain of *A. flavus* that does not produce aflatoxin. When AF36 colonized wheat is spread on the soil surface there is a shift in the microbial ecology whereby AF36 predominates the *A. flavus* population. When AF36 infects developing cotton seed instead of other A. flavus strains there is a reduction in aflatoxin content of cottonseed. It is currently registered in Arizona and Texas.

Bacillus popilliae

Also known as milky spore disease, it is a pathogen of Japanese Beetle. Unlike *Bacillus thuringiensis* which has an endotoxin, *B. popilliae* is a parasite causing a disease in beetle larva.

Bacillus thuringiensis

There are several isolates and formulations of Bt. It is considered to have the largest market among individual biopesticide active ingredients. While IR-4 was not involved in the initial registration of this product, in 1976 the IR-4 Project submitted a petition which resulted in a blanket tolerance exemption for Bt in beeswax, honey and all other raw agricultural commodities and greatly expanded the uses of Bt and eliminated the need to apply for a tolerance exemption on a crop by crop basis.

Cinnamaldehyde

As the name implies this product is found in cinnamon, particularly cinnamon oil, but it is also present in other oils as well. The initial registration work by IR-4 was on mushrooms (*Verticillium* spot and dry bubble disease control) and was later expanded to insect, mite and disease control on 53 crops. This product has both pesticidal activity and activity as an attractant.

Codling Moth Granulosis Virus

This is an important tool in the protection of pome fruits from codling moth. The IR-4 submitted the registration and tolerance exemption package for this product. It is a baculovirus which infects the larvae. The protein coating of the virus is dissolved in the alkaline environment of the larval midgut. The virus is then released into the infected host.

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Formic Acid and Thymol

These two active ingredients are used for the control of varroa mite which is a parasite of honeybees. These substances are volatile and fumes disseminate throughout the hive. Formic acid is also part of honeybee venom and thymol is a constituent of several herbs. The national registration for formic acid was submitted by IR-4. IR-4 also organized the current Section 18 for thymol.

Gibberillic Acid

There are several forms of this plant growth regulator which is also known as GA. The form GA_3 is the most widely utilized to increase plant elongation, fruit set and size. Similar to Bt, the IR-4 Project developed a blanket exemption from tolerance on all food crops.

Grape Berry Moth Pheromone

The active ingredient in this pheromone is (Z)-dodec-9-enyl acetate. It is a female sex pheromone that attracts males. Innundative releases of pheromone in vineyard air makes it harder for grape berry moth males to locate females thus reducing mating and egg production.

Kaolin

Kaolin is a type of clay. Fine white clay particles coat the trees and act as a protective barrier. The primary use is on pome fruit, especially for the control of pear psylla in the Pacific Northwest. In addition to insect and mite control, the white color tends to cool leaf and fruit surfaces reducing heat stress and sunburn.

Lagenidium giganteum

This is a fungus that controls mosquito larvae in rice fields and other bodies of fresh water. IR-4 submitted the tolerance petition for this product.

Lysophosphatidylethanolamine (LPE)

LPE is a product derived from egg yolks. It improves crop quality and accelerates ripening, and increases shelf life of stored crops and cut flowers by

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inhibiting an enzyme that causes aging and deterioration. When used as a product applied to fruit trees, LPE increases the rate of ripening by promoting the plant to produce more ethylene, which is an endogenous ripening substance. LPE is also has use as a postharvest storage product, inhibiting one of the major enzymes that breaks down membrane phospholipids. By inhibiting this enzyme and thereby helping to keep the membranes healthy, LPE increases the shelf life of stored produce and cut flowers.

Methyl Anthranilate

This is a natural product which was originally isolated from grapes. It repels many kinds of birds, including geese, gulls, blackbirds, crows, and starlings

Milsana

Milsana is an extract of giant knotweed *(Reynoutria sachanilensis*) which activates plant defenses against powdery mildew and other diseases. It also intensifies green leaf color. While it is currently labeled only in ornamentals, IR-4 has funded efficacy studies and recently submitted a request for an exemption from tolerance in food crops.

Spinosad

Spinosad is a mixture of spinosyns A and D which are fermentation products of the organism *Saccharopolyspora spinosa.* This product is not considered a biopesticide by EPA but does have an organic formulation. While the initial registration was handled by the registrant, the IR-4 Project developed logical associations among various crops and crop groups (*7*) which greatly reduced the number of residue trials needed to establish tolerances. IR-4 has conducted many residue trials and submitted them to EPA. Some specialty crops were also included based on extensions of data developed by the registrant. This unique approach which was approved by EPA (*8*) was largely facilitated by the reduced risk status and favorable environmental attributes of this product in combination with a comprehensive research plan *(9*) that created a large database of residue studies. This has led to its registration on over 200 specialty crops.

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Sucrose octanoate

This is a fatty acid ester of the sugar sucrose. It appears that this product has surfactant-like qualities causing suffocation or dewaxing of the insect cuticle resulting in dessication of insects. It was developed through the USDA-ARS Appalachian Fruit Research Station. It has activity on soft bodied insects and mites and is also marketed for varroa mite control in bees.

Verticillium WCS 850 *(V. albo-atrum)*

Current evidence indicates that this species is actually *albo-atrum*. This is a hyaline mutant and therefore does not produce microsclerotia and does not persist in the environment. It is injected into the trunk of American elm causing induced systemic resistance against Dutch Elm disease. It is currently under an Experimental Use Permit.

Yeast hydrolysate

Yeast extract hydrolysate is from *Saccharomyces cerevisiae*, and is also known as brewers yeast. It induces resistance in treated plants against bacterial and fungal diseases. It was sold as part of a fertilizer product for many years until the disease control abilities were recognized. It appears to act by enhancing the plant's natural defense mechanisms. The active ingredient is approved for use on all food crops, as well as on turf and ornamental plants, but is only currently marketed in citrus and tomato.

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Chapter 5

Organic Pesticide Use: What We Know and Don't Know about Use, Toxicity, and Environmental Impacts

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Organic farmers are allowed to use an array of chemicals and natural substances as pesticides. Yet data on the actual use of approved pesticides on organic farms are virtually non-existent. What little data exist for the use of pesticides approved in organic farming are for all farms, not just organic farms. As organic foods continue to gain wider consumer acceptance and as the acreage devoted to organic food and fiber production increases, more accurate and complete accounting of pesticide use on organic farms is needed. A more complete accounting of the toxicology of the botanical, synthetic, and other pesticides approved for use by organic farmers is warranted. Preliminary analysis indicates that environmental risks of some organic pesticides may be greater than the risks posed by synthetic pesticides. This highlights the need for better methods of assessing the environmental risks of pesticides and for comparing the risks posed by different pest management strategies.

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
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Despite the common misperception that organic farming is "pesticide-free", organic farmers are allowed under current and past rules to use an array of synthetic chemicals, botanical extracts, minerals, soaps, bacteria, and clays as pesticides. Yet data on the actual use of approved pesticides on commercial organic farms are virtually non-existent. This is likely due to multiple factors, including the large number of independent organizations that have historically each set their own standards for defining "organic" as well as the comparatively small acreage devoted to organic production.

Still, the comparative lack of data on organic farm pesticide use is surprising given the amount of information potentially available. Most organic certification organizations at the state, national, and international levels have required participating organic farmers to submit detailed reports of the pesticides that were used and/or are planned for use on the farm. There have been independent organic certification groups working at both the state and national levels for over 30 years. Yet despite such extensive, long-term reporting to certification agents, essentially no publicly available data currently exist regarding pesticide use on organic farms.

Considering that organic is reported to be the fastest growing sector of the food industry and as the amount of acres under organic management increases, better knowledge and statistics about the use of pesticides on organic farms should now be important.

This is especially true in light of the environmentally persistent nature and toxicity of the copper-based fungicides used widely by organic farmers. Furthermore, little is known about the toxicity profiles and overall consumer exposures to many of the widely used organic botanical insecticides. A residue test method exists for only one organic-approved insecticide, pyrethrum, and only because there are synthetic analogs of pyrethrum that the government routinely tests for.

Researchers with the Organic Materials Review Institute and Consumers Union have remarked on the comparative lack of safety and residue data for organic pesticides, stating "the lack of residue data . . . and the lack of complete toxicological data for most [organic] insecticides, have seriously limited ability to carry out risk assessments for these pest management products. . . . It seems essential that the widely used [botanical organic pesticides] be more completely tested for the full range of toxic effects that conventional pesticides are currently tested for. Expanded efforts to collect data on possible residues of the natural pesticides in organic and non-organic foods are also needed. Better toxicity data and residue data will improve the basis for risk assessments of these pest-management tools (*1*)."

The creation of the US Department of Agriculture's National Organic Program (NOP) in 2000 has unified the rules for organic farming nationally in the United States and provides an important new opportunity for collecting comprehensive data on organic farm pesticide use at the national level.

Organic Pesticides: What We Know

What is an organic pesticide? In conceptual terms, almost any natural substance can be used as an organic pesticide. Many people are surprised to learn that the US Department of Agriculture does not keep or maintain a list of approved organic pesticides. Nor does the USDA maintain a list of organic approved pesticide active ingredients. Instead, the USDA NOP maintains a "National List" of prohibited natural substances and approved synthetic chemicals (*2*). If a natural chemical or substance is *not* on the National List, it can legally be used as an organic pesticide.

In practical terms, there are a relatively limited number of active ingredients that are used as pesticides in commercial organic farming. The main organic pesticides include horticultural oils (derived from both refined petroleum and botanical sources), sulfur, copper compounds, the soil bacterium *Bacillus thuringiensis* (Bt), pyrethrum, rotenone, neem, and spinosad. These pesticides are used as insecticides, fungicides, and bacteriocides.

A few citric and acetic acid-based organic herbicides have recently gained approval by the U.S. National Organic Standards Board (NOSB) for use in organic farming. While their use by commercial organic farmers is likely limited due to their relatively high cost, lack of effectiveness, and lack of selectivity, data on their use would nonetheless be informative for policymakers.

While there are scant data on pesticide use on organic farms, there is some data available on the use by all farmers of some organic-approved pesticides. Oil and sulfur, for example, were the two most heavily used pesticides in the United States as of 1997 (based on total pounds of active ingredient applied to U.S. crops) (*3*). More than 100 million pounds of oil and nearly 80 million pounds of sulfur were applied in 1997 according to estimates by the National Center for Food and Agricultural Policy (NCFAP), the latest year for which comprehensive data exists. Oil accounted for 56 percent of all pounds of insecticides applied to US crops. Sulfur accounted for 59 percent of all pounds of fungicide applied to US crops.

While most of this oil and sulfur was applied on non-organic farms, the relatively high application rates of these organic-approved pesticides indicate that as the number of cropped acres under organic management increases, total pounds of pesticide active ingredient applied may increase significantly.

Perhaps the most heavily used organic pesticide is Bt. However, Bt is not quantifiable in terms of pounds of active ingredient applied per acre because it is sprayed as a solution of live bacteria and crystallized toxin protein. However, the number of acres treated with Bt sprays is quite large and likely represents the organic pesticide used to treat the most cropped area on organic farms.

In fact the heavy dependence upon sprayed Bt insecticides by organic farmers raises concerns of increased pest resistance to Bt. Several cases of resistance to sprayed Bt insecticides have been documented, whereas pest

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resistance to genetically modified Bt crops where the toxin protein is expressed in the plant tissues has not developed despite the significantly larger crop area devoted to biotech Bt crops (*4, 5*).

Organic-approved copper, in the form of various copper-based fungicides, was the 18th most-heavily used pesticide active ingredient and the second most heavily used fungicide in the US, according to NCFAP data for 1997. Some 13.6 million pounds of copper-based fungicides were applied to more than 50 different crops on more than 3 million acres.

Copper is a broadly toxic, persistent element whose use as a pesticide has led to liver disease in farm workers and possible cancer (*6, 7*). Moreover, continued use of copper-based fungicides has led to phytotoxicity in crops due to the high soil copper levels (*8*). Because of its environmental persistence and comparatively high toxicity, the European Union was slated to ban all copper-based fungicides in early 2002. In anticipation of the ban, the EU funded considerable research across Europe into an organically acceptable and viable alternative fungal disease control strategy beginning in 1998. However, this research failed to develop a viable alternative by the planned 2002 phase-out date, so the ban on copper-based pesticides has since been delayed indefinitely until organic-acceptable alternatives have been developed (*9*).

Because of their high application rates, oil, sulfur, and copper accounted for fully 25 percent of the total pounds of all pesticide active ingredient applied to US crops in 1997. While these figures are for both organic and non-organic farms, the application rates of these pesticides are nominally based on their effective use rates and can thus be inferred as a reasonable estimate of the application rates of these pesticides on organic farms.

The average application rate of sulfur was 35 lbs per acre versus an average synthetic fungicide application rate of only 1.6 lbs per acre. Thus, sulfur was applied at more than 20 times the average application rate of synthetic fungicides. Copper was applied at an average rate of over 4 lbs per acre on an estimated 3.3 million acres of crops. This is an application rate more than 2.5 times higher than the synthetic fungicide average.

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Organic Pesticides: What We Don't Know

Unfortunately, it is simply unknown how much oil, sulfur, and copper are used on organic farms, on which crops, and at what application rates and frequencies. It may be that the statistics from both organic and non-organic farms underrepresent the use of these pesticides on organic farms. Many non-organic farmers use copper, sulfur, and oil in combination with other, more effective, lower use rate synthetic pesticides. As such, this may skew the statistics toward lower use rates than may be common on organic farms. It may be that organic farmers use very little of these pesticides and instead are making up for reduced crop yields through the higher prices for organic crops. Or it may be a combination of these two.

As for the other commonly used organic pesticides—such as pyrethrins, neem, rotenone, sabadilla, and the newest organic-approved pesticide, spinosad—there is little information available about their use on organic farms. The few data that are available do not delineate use on organic and non-organic farms. There is no information at all on the application rates, frequency of application, and the range of crops on which organic farmers use these organic-approved pesticides.

The USDA simply doesn't keep statistics for these pesticides. The California Department of Pesticide Regulation (CDPR) maintains a database ostensibly of all pesticides used by growers, professional applicators, and/or exterminators in that state. This database contains data on several organic-approved pesticides (10).

These data indicate California use in 2002 of 53 million pounds of sulfur, over 18 million pounds of petroleum oil, 5 million pounds of mineral oil, 160,000 pounds of cottonseed oil, 110,000 pounds of vegetable oil, 430,000 pounds of kaolin, 2.9 million pounds of copper sulfate (pentahydrate), over 876,000 pounds of copper sulfate (basic), 326,000 pounds of copper, and 59,000 pounds of copper oxychloride. These total over 80 million pounds of organic-approved active ingredients applied to cropland in 2002. What proportion of this was applied on organic farms is unknown.

The CDPR indicates that 55,000 lbs of spinosad were applied to cropland in 2002. However, because spinosad did not gain organic approval until 2002, it is unclear how much if any of this was used on California organic farms.

Finally, the CDPR database indicates use of 5,000 pounds of diatomaceous earth, over 5,000 pounds of pyrethrins, 165,000 pounds of potash soap, over 300,000 pounds of neem oil, 385 pounds of rotenone, over 680 pounds of garlic, and over 250 pounds of sabadilla extract. This roughly half a million pounds of organic pesticides are likely to have been used mostly if not entirely on organic farms because non-organic farmers have more effective and cheaper alternatives to these botanical/natural products.

More detailed information on the use of pesticides on organic farms could be obtained through an examination of organic farm records. Organic farmers are required to submit detailed Organic System Plans (OSPs) each year to their organic certifying agent, who is charged by the USDA with inspecting both the farm and its records annually. The OSPs are supposed to include a listing of any pesticides used or planned for use on the farm. This listing requirement applies to all pesticides used, including organic-approved pesticides.

It would seem to be relatively easy to use these OSPs to compile reasonably accurate data on the use of pesticides on certified organic farms. However, farmers must often respond to unexpected pest outbreaks and use pesticides that were not listed in the OSP. While farmers are supposed to inform certification

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agents of past pesticide use, it is unclear how commonly this is done, especially for pesticides used to quell unexpected pest outbreaks. Moreover, the OSPs are considered confidential business records and are protected from outside review by the public. Not even the certification agent can release information from the OSP to the public.

So far, no state-level or national government agencies have used these records to compile more accurate and complete statistics on the use of pesticides on organic farms. Perhaps a university research group could obtain permission to glean such data from the OSPs after agreeing not to disclose confidential business information and under the supervision of the certification organization. It is clearly an area warranting further study and the OSPs could prove a valuable resource.

Some may ask whether it is necessary to gather more accurate statistics on the use of pesticides on organic farms. After all, organic pesticides are approved by the USDA, EPA, and FDA. Many organic pesticides, such as botanical insecticides, are relatively low-toxicity compounds that readily degrade in the environment. However, the same arguments can be made about synthetic pesticides and yet detailed statistics are gathered to inform the public, regulators, and policy makers of their use and potential risks. No matter what the origin of a pesticide, it is wise to have an accurate accounting of how it is used in agriculture, including in organic farming.

Not only are there human safety concerns, but there are ecological concerns as well, especially in regard to copper-based organic pesticides. Many of the botanical organic pesticides have not been fully characterized for their human and ecological toxicity (1). Moreover, even the lower-toxicity organic pesticides have comparatively high application rates and frequencies that warrant a more comprehensive risk assessment for policy makers, regulators, and consumers—many of whom mistakenly believe that no pesticides are used by organic farmers.

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Assessing Toxicity and Environmental Impacts

While there is a lack of detailed information on the on-farm use of pesticides by organic farmers, somewhat more effort has been placed upon assessing the potential environmental impacts of pesticide use in organic farm systems compared to non-organic farming systems. The relative toxicities and their impacts can be explored by looking at case studies of particular crops and pesticide regimes.

In April of 2001, the journal *Nature* published a paper by researchers from Washington State University purportedly demonstrating that an organic apple production system "gave similar yields" as a conventional growing system but was far more sustainable and had far less negative environmental impact than conventional apple production. Specifically, Reganold et al. (11) concluded that the conventional apple production system had an environmental impact 6.2 times higher than the organic system based on an assessment of pesticide use with one type of environmental impact index. Reganold et al.'s results were widely covered in the national and international media as scientific evidence that organic farming is far less environmentally damaging than so-called "conventional" farming.

However, a critical review of Reaganold et al. (11) and a comparison of their environmental impact assessment with results obtained from other environmental impact assessment formulae indicated that the analyses can be highly subjective, depending on the environmental risks accounted for and the weighting applied to the measured parameters. For example, it will be shown that analyzing Reganold et al.'s (11) data with an environmental impact assessment method developed at Cornell University, for example, resulted in similar environmental impact scores between the organic and conventional systems, rather than a six-fold difference. Thus, a detailed examination of the ways in which these rating systems guage potential environmental impact is valuable in placing the conclusions in their proper context and recognizing their limitations and biases.

Reganold et al. (11) examined the environmental impact of three different apple production systems—organic, conventional, and integrated pest management (IPM)—using a formula developed by Stemilt Growers, Inc. of Wenatchee, Washington. Stemilt devised its "Responsible Choice" rating index to help guide tree fruit farmers in choosing low impact pest-control options and as an 'ecolabeling' marketing tool to influence consumer purchasing decisions (*12*).

The Stemilt Responsible Choice (RC) system rates the potential for environmental impacts of pesticides based on eight basic parameters (Table I) and applies the cumulative points score for a pesticide based on the labeled application rate, rather than the amount (i.e., dose) of application per unit area.

The Stemilt RC pesticide score formula is:

$$\text{RC Score} = (3 \times \text{SE}) + \text{D} + (2 \times \text{PI}) + \text{SS} + (2 \times \text{LP}) + \text{SL} + \text{BD} + \text{B} \quad (1)$$

Spray efficacy (SE) is a measure of the effectiveness of a pesticide *against a specific target pest*, resulting in RC scores for a specific pesticide that vary depending on the targeted pest. Dermal LD_{50} (D) is a measure of farmworker toxicity. The preharvest interval (PI) is a measure of consumer safety (longer = more points). Soil sorption (SS), half life (SL), and leaching potential (LP) are measures of environmental contamination and persistence. Biological disruption (BD) is an estimate of the long-term impacts on beneficial insects in a field. The

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effect on beneficials (B) is a measure of the short-term toxicity to insects in the field.

Each of these eight parameters is scored based on the scales listed in Table I. The RC scores for a pesticide can range from a low of 6.5 points, to a theoretical high of 62.3 points. An example of the RC scores for conventional insecticides and the organic approved pheromone-based mating disruption is shown in Table II.

Efficacy, the most heavily weighted factor in the RC formula, is based on the effectiveness of a pesticide against a specific target pest. Thus, Stemilt RC scores for pesticides change depending on the pest targeted in a specific application. In other words, the same pesticide sprayed at the same application rate against different pests will have different RC scores depending on the efficacy of that pesticide against each target pest.

Clearly the RC scoring system encourages farmers to use the most effective pesticides against specific pests. Less clear, however, is why efficacy of a pesticide should bear on an assessment of that pesticide's environmental impacts. After all, the environmental impacts of spraying two pounds per acre of pesticide X will be the same no matter how effective it is against any given target pest.

RC scores apply to the labeled use rate of a pesticide (*13*). However, in their paper in *Nature*, Reganold et al. adjusted the Stemilt RC scores for each chemical to reflect the RC points per "unit amount" of applied pesticide (kilogram or liter), apparently to satisfy the requirements of *Nature's* editors (*14*). In so doing, the authors adjusted the RC scores per acre into hectares by multiplying by 2.471 and then dividing by the number of units (liters or kilograms) applied to obtain the RC points per unit. Thus, pheromone mating disruption, which has an RC score of 6 points per acre, works out to 14.8 points per hectare, or 0.015 points per unit (6 x 2.471 ÷ 988 ties = 0.015). Regardless of whether or not the scores were adjusted to reflect the larger unit area (acres vs. hectares), the ratio of the scores between any two farming systems would remain the same.

An examination of the values assigned to certain pesticides revealed that Reganold et al. may have used incorrect Stemilt RC scores. For example, glyphosate has a Stemilt RC score against most weeds of 9.3 points per application when applied at the label rate of 3-7 L/ha (0.32-0.75 gal/A) (*13*). However, Reganold et al. list glyphosate as 9.3 points *per liter* when applied at the rate of 4.7 liters per hectare, for a total of 43.71 points per application. Dividing the 43.71 points per hectare by 2.471 (to give the total points per acre) gives 17.7 RC points per acre application rather than 9.3 points.

If the 9.3 points per acre labeled rate of application is correct, then multiplying by 2.471 to convert acres to hectares gives 23 points per application. The RC score total for glyphosate in the conventional system would then be 529 points, rather than 1,022 as calculated by Reganold et al. (*11*). The total

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Table 1. Responsible Choice Rating System

Factor	*Coefficient*	*Rating Points*	*Total Possible RC Points*	*Source*
Spray Efficacy (SE)	3	1-4 (1 = most efficacious)	3-12	WSU Tree Fruit Spray Guide
Dermal LD_{50} (D)	1	<500 = 2.5 500-1,000 = 2.0 1001-2000 = 1.5 2001-5000 = 1.0 >5000 = 0.5	0.5-2.5	EPA registration
Preharvest Interval (PI)	2	# days/7	0-8	Pesticide Label
Soil Sorption (SS)	1	KOC values <500 = 1.75 501-1000 = 1.5 1001-2000 = 1.25 >2000 = 1.0	1.0-1.75	WSU water quality guide
Leaching Potential (LP)	2	Small risk = 1 Medium risk = 2 Large risk = 3	2-6	WSU water quality guide
Soil Half-Life (SL)	1	# days/20	0-2	WSU water quality guide and registration data

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Biological Disruption (BD)	1	0-25	0-25	Fieldman observation (Used for Penn Cap M only)
Effect on Beneficials (B)	1	0-5	0-5	WSU Tree Fruit Spray Guide

SOURCE: Stemilt Growers, Inc., Wenatchee, WA

NOTE: WSU is Washington State University; Penn Cap M is a microencapsulated formulation of methyl parathion

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Table II. Responsible Choice (RC) Scores for Insecticides Used in Codling Moth Control

Pesticide	*SE*	*D*	*LP*	*SS*	*PHI*	*SL*	*B*	*BD*	*Total*
Mating Disruption	3-4 (1.5)	2,000 (1.5)	NA (0)	NA (0)	0 (0)	NA (0)	0 (0)	0 (0)	6.0
Phosmet	4 (1.0)	>4,640 (1.0)	Small (1.0)	612 (2.5)	7 (1.0)	12 (0.6)	(2.0)	(0)	13.1
Azinphos-Methyl	4 (1.0)	220 (2.5)	Small (1.0)	1,000 (2.0)	7 (1.0)	40 (2.0)	(2.5)	(0)	16.0
Methyl Parathion	3 (2.0)	5,400 (0.5)	Small (1.0)	5,100 (1.0)	14 (2.0)	5 (0.25)	(5.0)	(25.0)	43.8

SOURCE: Stemilt Growers, Inc., Wenatchee, WA

NOTE: The factor abbreviations and associated units are given in Table I. Numbers on top are the characteristic values for the selected factor, and numbers in parentheses indicate the chemical's score for each factor in the RC formula. Total points equal the sum of the factor coefficient multiplied by the factor score.

conventional score under the RC formula is then 2,399 points, rather than 2,893, a reduction of 17 percent. (Recalculated RC values are shown in Table V.)

Stemilt personnel purportedly supplied Reaganold et al. with an RC score for glyphosate of 17 points per application at the label rate and indicated that RC scores for pesticides have changed over time as new information has become available (*15*). Unfortunately, Stemilt does not publish nor make publicly available its current or past RC ratings for pesticides, so it is difficult to determine what the appropriate score for glyphosate should be in this instance. Pertinently, the target weeds glyphosate was used against in the study were not identified, and, therefore, the appropriate efficacy factor to put into the RC formula would be unknown.

The above examples of scoring for glyphosate illustrate the potential problems of environmental rating systems like the RC system. Scores vary depending on the target pest, and subjective information leads to changes in RC scores over time. Analysis of potential problems in deriving an impact score using the Stemilt RC system raises the question of how other environmental impact indices would comparatively rate farming practices.

The Environmental Impact Quotient (EIQ) developed at Cornell University seems a more comprehensive and consistent environmental impact rating system for agricultural pesticides than the RC system (16). Like the Stemilt formula, the Cornell EIQ was devised in the early 1990s as a tool to help farmers and researchers assess the potential environmental impacts of pesticide use, with an eye toward encouraging farmers to choose less harmful pesticides as part of their Integrated Pest Management programs. And like Stemilt's RC formula, the Cornell EIQ factors in known toxicological and environmental parameters of a pesticide to obtain an EIQ score.

However, unlike Stemilt's RC formula, the EIQ score for a pesticide is converted into a Field Use Rating based on the dose of pesticide application. Thus, the EIQ system accounts for application rate differences among pesticides and encourages the use of lower-rate pesticides, all other factors being equal. Environmentally, this makes more sense than Stemilt's RC formula, which focuses more on agronomic impacts of pesticide use.

The Cornell EIQ formula also takes into account a far more comprehensive set of chemical characteristics and potential environmental impacts than does the Stemilt RC formula. In addition to factoring impacts on farm workers, consumers, and beneficial insects, the Cornell EIQ accounts for potential impacts on bees, fish, and birds. In all, 11 different factors are used to calculate risks to pesticide applicators, pickers, consumers (both food residues and groundwater), aquatic organisms, birds, bees, and beneficial arthropods (Table III).

The Cornell EIQ score is calculated by the following formula (see Table III for definition of variables):

$$\mathrm{EIQ} = \{C[(DT \times 5) + (DT \times P)] + [(C \times ((S + P)/2 \times SY) + (L)] + [(F \times R) + (D \times ((S + P)/2) \times 3) + (Z \times P \times 3) + (B \times P \times 5)]\}/3 \qquad (2)$$

The EIQ formula consists of three parts, a farmworker component, a consumer component, and an ecological component. Each of these three components is given equal weight in the final analysis, but within each component, individual factors are weighted differently.

The farm worker risk is the sum of the applicator exposure rating (DT x 5) plus picker exposure rating (DT x P) times the long-term health effect or chronic toxicity rating (C). The factor of five applied to the dermal toxicity rating in the farm worker risk component is to account for the higher risk from handling concentrated pesticides. The picker component multiplies the dermal toxicity rating by the score for plant surface residue half-life.

The consumer component is the sum of the consumer exposure ratings (C x ((S + P)/2) x SY) plus the potential groundwater effect score (L).

The ecological component is the sum of the effects on fish (F x R), birds (D x ((S + P)/2) x 3), bees (Z x P x 3), and beneficial arthropods (B x P x 5). Because terrestrial organisms are more likely to be in agricultural areas, greater weighting is applied to them (3) than to fish (1). The impact on beneficials is

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Table III. Cornell EIQ Pesticide Rating System

		Rating Score Criteria		
Variable	*Symbol*	*1*	*3*	*5*
Chronic toxicity	C	Little or none	Possible	Definite
Acute dermal (LD_{50} for rabbits/rats, mg/kg)	DT	>2000	200-2,000	0-200
Bird toxicity (8 day LC_{50})	D	>1,000 ppm	100-1,000 ppm	1-100 ppm
Lethality to bees (field doses)	Z	Relatively non-toxic	Moderately toxic	Highly toxic
Beneficial arthropod toxicity	B	Low impact	Moderate impact or post-emergent herbicides	Severe impact
Fish toxicity (96 hr LC_{50})	F	>10 ppm	1-10 ppm	<1 ppm
Soil residue half-life	S	<30 days	30-100 days	>100 days
Plant surface residue half-life	P	1-2 weeks	2-4 weeks	>4 weeks
Mode of action or Systemicity	SY	Non-systemic and all herbicides	Systemic	
Leaching potential (water half-life, solubility, adsorption, soil properties)	L	Small	Medium	Large
Surface loss potential (water half-life, solubility, adsorption, soil properties)	R	Small	Medium	Large

Note: 1 = least toxic or harmful, 5 = most toxic or harmful. Source: Cornell University.

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given the greatest weighting (5) because these organisms likely spend significant amounts of time in fields. Wildlife mammalian toxicity is not included in the ecological component because it is already accounted for in the farmworker risk component, calculated based on animal test data.

To compare environmental impacts of pesticide use among different farming systems, the EIQ scores for each pesticide must be converted from individual pesticide ratings into pesticide field use ratings as follows:

EIQ Field Use Rating = EIQ x % active ingredient x Application Rate (lb/A) (3)

The EIQ system socre is obtained by summing the field use ratings for all pesticides used. For example, a chemical with an EIQ score of 10, which is 20% active ingredient, and is applied at two kilograms per hectare would result in an EIQ field use rating of 10 x 0.2 x 2 = 4. Adding up all of the field use ratings for all the pesticide inputs used over the course of the growing season would give a cumulative EIQ system score that can then compared with EIQ system scores of other pesticide regimens.

Application of the EIQ formula methodology to the organic apple production system examined by Reganold et al. (11) resulted in a score of 7,396 points versus the conventional system score of 9,790. (Tables IV, V). The Cornell EIQ formula thus scores the conventional system only 32 percent higher than the organic system, whereas the RC formula scored the conventional system 520 percent higher than the organic.

Why do two different scoring systems yield such a large difference in environmental impact ratings? Firstly, Stemilt's RC scoring system is designed to encourage farmers to use pesticides that are the most compatible with biological pest control. As such, the RC formula assesses a heavy point penalty on pesticides that can potentially harm beneficial organisms, adding up to 30 points out of a possible total of 62.3 points. Thus, impact on beneficials can account for nearly half of a chemical's total RC score. In contrast, the Cornell EIQ simply rates the potential environmental impacts of a pesticide based on its toxicity and chemical characteristics, irrespective of compatibility with biological control methods.

Table IV. Environmental Impact Rating Differences

Rating Formula	*Conventional*	*Organic*	*Ratio Conventional/Organic*
Stemilt Responsible Choice	*2,893.1	465.6	6.21
Cornell EIQ	9,790.1	7,396.0	1.32

Note: RC scores are as published in (11). EIQ scores were calculated according to Kovach et al. (16); details are shown in Table V.

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Table V. Comparison of environmental impact scores using the Stemilt Responsible Choice (RC) formula and the Cornell EIQ formula.

Pesticide	*# of sprays (5-year totals)*	*Rate of app. per hectare (product, not active ingredient)*	*Active Ingredient Content*	*Stemilt RC chemical rating per unit*	*Stemilt RC system score*	*Cornell EIQ chemical rating*	*Cornell EIQ field use ratings*	*Revised EIQ field use ratings (Washington state average use rates)(17)*
Organic system								
Bt	5	2.2 kg		7.28	81.5	13.5	148.5	
Pheromone [b]	4	988 ties		0.015	59.3	--	(59.3)	
Oil	5	18.7 liters/ 15.9 kg	0.98	0.42	39.5	27.5	2,142.5 [a]	4,285 (2 sprays/year)
Sulfur	11	11.2 kg	0.90	2.32	285.3	45.5	5,045.6 [a]	7,796.9 (3.4 sprays/year)
Organic System Total					465.6		7,396	12,289.7
Conventional system								
Bt	4	2.2 kg		7.28	65.2	13.5	118.8	
Oil	5	18.7 liters/ 15.9 kg	0.98	0.42	39.5	27.5	2,142.5 a	
Sulfur	9	11.2 kg		2.32	244.5	45.5	4,127.7 [a]	
Pheromone [b]	4	988 ties		0.015	59.2	--	(59.2)	
Azinphos methyl	16	2.2 kg	0.50	17.64	632.3	43.1	758.6 [a]	

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Captan	1	14 liters		4.02	56.3	28.6	400.4	
Carbaryl	2	1.8 liters		13.53	47.4	22.6	81.4	
Chlorpyrifos	7	1.2 liters/ 1.28 kg	0.50	10.57	86.5	52.8	236.5[a]	
Ethephon[b]	7	2.1 liters		2.33	34.2	9.3[§]	136.7	
Fenarimol	1	0.4 liters		83.52	33.4	27.3	10.9	
Glyphosate	23	4.7 liters/ 4 kg	0.41	4.89	528.5	32.4	1,222[a]	478 (1.8 sprays/year)
Imidacloprid	3	0.1 liters		274.84	57.8	37.2	11.8	
Myclobutanil	7	0.4 liters		131.92	323.7	41.2	115.4	
Norflurazon	3	2.2 kg		10.81	90.8	18.8	124.1	
Triflumizole	4	0.6 kg		18.7	41.9	56.6	135.8	
Simazine	3	2.3 liters		8.25	57.8	15.7	108.3	
Conventional System Total					2,398.5		9,790	9,046

NOTE: Score for glyphosate has been adjusted to reflect accurate Stemilt RC score of 9.3 points per application at label rate.

[a] The field use ratings for these chemicals have been calculated using the percent of active ingredient. For the other pesticides, the specific formulation was not known and so the active ingredient percentages were assumed to be 100%. This overestimates the Cornell EIQ field use ratings for these pesticides.

[b] An EIQ for ethephon was not included in the original group of pesticides calculated by Cornell University. The EIQ for ethephon was calculated using the EIQ formula and verified by Dr. Kovach. An EIQ for pheromones was not calculated because pheromones were used equally in all treatments, balancing the score for this input and because it is unclear how an EIQ would be calculated for a product that is not applied.

Secondly, the RC formula does not account for the application rates of pesticides. RC scores apply to the labeled use rate of a pesticide, but these use rates never factor into the formula. Therefore, two pesticides with identical toxicology and environmental profiles will receive the same RC score, even if the label application rate on one pesticide is ten pounds per acre and the other only one pound per acre. This specific aspects of the RC formula heavily favor high application rate pesticides, such as organic-approved oil and sulfur, over low rate synthetic pesticides. In contrast, the Cornell EIQ factors dose and application frequency into its formula, thereby accounting for one of the central tenets of toxicology: the dose makes the poison.

As can be seen from Table V, this system of environmental impact assessment results in significantly different conclusions compared to the Stemilt RC system. For example, whereas oil accounts for 8.5 percent of the organic system score using the RC formula (39.5 points out of 465.6), oil represents 29 percent of the organic system score using the Cornell EIQ formula (2,142.5 points out of 7,396).

Under the RC system, the organic-approved pesticides used in the conventional system (Bt, oil, sulfur, and pheromone) accounted for only 14 percent of the total score, whereas under the EIQ system, the organic-approved inputs used in the conventional system accounted for 66 percent of the total system score.

The differences between the RC and Cornell scores are mostly a result of the Cornell formula factoring in application rates and percentage active ingredient of the chemicals, as well as the more comprehensive set of risk factors accounted for in the EIQ formula.

The major differences in conclusions between the Cornell EIQ formula and the Stemilt RC formula can best be demonstrated in a comparison of the field use ratings for two pesticides under each rating system. The Stemilt RC system scores each application of sulfur at the label rate of 10 lbs per acre at 10.1 points (RC score for micronized sulfur against mildew in apples applied at 10 lbs per acre, which is equal to 11.2 kg per hectare, or 2.32 RC points per kg as seen in Table 5) and each application of glyphosate at the label rate at 9.3 points (although as discussed earlier, Reganold et al. used a score of 17 points per acre application at the label rate). In contrast, the Cornell system rates each application of sulfur at 458.6 points (45.5 points x 0.90 % active ingredient x 11.2 kg) and each application of glyphosate at 53.1 points (32.4 points x 0.41 % active ingredient x 4 kg).

Thus, the score ratio between sulfur and glyphosate is 1.08 under Stemilt's RC system (10.1:9.3), compared to 8.63 for the Cornell system (458.6:53.1). That is an eight-fold difference in score ratio.

What is perhaps most interesting about Reganold et al.'s comparison is the treatment differences among the organic and conventional plots within the 4.2 acre research orchard. Reganold et al. reported "there were no observable

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differences in pests, disease or physiological disorders among plots during each growing season" (*11*). Yet the conventional plot received nearly 85% of the organic pesticide applications, plus 11 additional applications per year of synthetic insecticides and fungicides (for a total of 55 additional pesticide sprays). Were the extra pesticide treatments in the conventional plots really necessary given that organic plot yields were 93 percent of the yields of the conventional plots? Reganold et al. have said they were necessary in correspondence with the editors of *Nature* in response to a letter from this author. Reganold et al. stated that the higher pesticide treatment levels on the conventional plots were based on "the advice of the professional pest consultants, who regularly monitored pest pressures and made spray recommendations to the grower" for both the organic and conventional systems (*18*). However, this statement is somewhat confusing given their statement that "there were no observable differences in pests, disease or physiological disorders among plots during each growing season."

Unfortunately, control plots—apple trees with no pesticides applied, or conventional plots sprayed only with oil and sulphur—were not incorporated into the experimental design. Therefore, no conclusions can be drawn as to whether organic methods truly obviate pest damage or reduce the need to use additional pesticides.

Importantly, glyphosate accounted for 35 percent of the conventional system's RC score, yet only 12 percent of the EIQ formula score. Glyphosate was applied on the conventional apple plots at an average of 4.6 times per season. However, in 2000, conventional Washington apple growers who used glyphosate averaged only 1.8 sprays per season. It is unclear why glyphosate needed to be applied 2.5 times more than the Washington state average, although it increased the conventional system RC score by roughly 20 percent.

Similarly, the organic apple plots received fewer than average number of sulfur and oil sprays compared to organic apple producers surveyed by Washington State University (Table VI). When the use of both the organic inputs and glyphosate are adjusted to actual grower application frequencies, the organic system scores 36 percent worse for the environment than the conventional under the Cornell EIQ rating system. (See last column of Table V).

This comparison of the Stemilt RC and Cornell EIQ rating systems using data from Reganold et al. demonstrates that environmental impact rating systems are highly subjective. The results depend heavily on the intended purpose of the rating system and the factors and weightings of the formula. While both the Stemilt RC and Cornell EIQ systems could help farmers lower their potential environmental impacts, it appears that the Cornell EIQ formula is more appropriate for assessing the potential off-farm impacts of pesticide use

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Table VI. Washington State 2000 Organic Producer Survey

Organic Grower	*Sulfur*	*Bt*	*Oil*	*Pheromone*
Grower #1	2	1	1	200 dispensers/acre
Grower #2	6	5	1	200 dispensers/acre
Grower #3	6	3	3	227 dispensers/acre
Grower #4	2	4	4	200 dispensers/acre
Grower #5	1	2	1	400 dispensers/acre
Total	17	15	10	
Average	**3.4**	**3.0**	**2.0**	**245 dispensers/acre**

Source: J Brunner, Washington State University, Tree Fruit Research & Extension Center.

because it accounts for potential risks to mammals, bees, birds, and fish. The RC formula only accounts for potential impacts on mammals and bees. Thus, certain pesticides used by organic farmers with higher risks for fish get a higher environmental impact score using the EIQ system. Moreover, the Cornell EIQ takes into account both the percentage of active ingredient and the application rates of pesticides and inputs, which is a more complete accounting for potential off-farm environmental risks.

Conclusion

As the acreage devoted to organic food and fiber production increases, there remains many unanswered questions about the amounts, toxicity, and environmental impacts of the pesticides used by organic growers. Many of these pesticides have not been thoroughly characterized for carcinogenicity and mutagenicity and consumer exposure information remains incomplete. It is clear that some organic pesticides pose potential environmental impacts as great or greater than their synthetic counterparts, especially fungicides. Moreover, an examination of the environmental and human health consequences of the production of botanical pesticides, such as pyrethrum, is warranted, as some of these utilize extensive acreage and human hand labor for their production.

The preceding discussion highlights some of the important information gaps and suggestions for future research. Hopefully, there will be significantly greater information available to future researchers examining these issues.

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Chapter 6

Putting the Toxicology and Risk Assessment of Approved Organic Pesticides in Perspective

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The U.S. Department of Agriculture (USDA) National Organic Program (NOP) is not a safety standard. The certified organic label does not mean that produce has been grown without the use of pesticides or that foods labeled as organic are more nutritious, safer, or of higher quality than foods produced by conventional agriculture. The NOP, which went into effect on October 21, 2002, provides marketing guidance on grower certification, methods and practices for organic food production and specifies which chemical substances can be used in organic food production and handling operations. NOP pesticides are subject to the same regulatory oversight as conventional pesticides and, if not used according to the labeled specifications, may pose significant risks to consumers, the environment, and workers. Allegations about environmental and food safety issues and exaggerations about the benefits of organic products have fueled misconceptions and misrepresentations about conventional agriculture. Consumers should have the option to choose between organic and conventionally grown foods, but this choice should be based on factual and not misleading information.

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

The assurance of a safe, affordable and plentiful food supply is a critical societal priority. Pesticide products play an important role in protecting crops and sustaining agricultural yields, regardless of whether farming practices are classified as conventional or certified as organic. Interest in organic farming is increasing. Before the 1990 Organic Foods Production Act, some states had no organic standards and some states and private organizations had established their own requirements and specifications for certifying organic products. These gaps and differences caused confusion and uncertainty among growers and consumers. The NOP has provided uniform and clear national standards for organic food production, processing and certification for the growers and producers of certified organic foods (*1*).

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Pesticide Benefits

The safe and judicious use of pesticide products provides significant benefits to agriculture and public health. Reducing competition from weeds and insect damage assures an affordable and abundant supply of fresh food, essential for good health and development. Preventing insect/rodent damage and the growth of deadly pathogenic molds and bacteria protects stored grain and other food supplies. The America Cancer Society (ACS) stated in *Unproven Risks: Pesticides* (*2*), "When properly controlled, the minimal risks they [pesticides] pose are greatly overshadowed by health benefits of a diverse diet rich in foods from plant sources" (*2*).

Effective insect and rodent control also averts a wide variety of diseases, including West Nile virus, other encephalitis, malaria, yellow fever, dengue fever, Hantavirus and bubonic plague (*3*). Herbicide use eliminates noxious weeds (poison ivy, oak and sumac) and potential breeding grounds for harmful insect pests.

The National List of NOP Approved Products

During the 1990's, the USDA convened an independent National Organics Standards Board to provide recommendations for the National List of Allowed and Prohibited Substances, also known as the National List (*4*). The National List represents a variety of substances that may or may not be used in organic food production and handling operations - inorganic and organic chemicals (e.g., sulfur, copper salts, crop oils, boron derivatives) and natural products (e.g., pyrethrum extracts, neem oil and rotenone). The NOP also allows the use of microbial insecticides, such as bacteria (e.g., various *Bacillus thuringiensis* (Bt) strains), fungi (e.g., *Beauvaria bassiana*), and viruses (e.g., *Baculovirus sp*) as well as various predatory organisms (e.g., ladybugs, parasitic wasps, lace bugs).

However, not all botanical insecticides (specifically extracts and active ingredients of tobacco [nicotine], sabidilla [veratidine]] and ryania wood [ryanodine]) that are approved for use in organic farming outside the United States are NOP-approved products.

The Growth of U.S. Organic Food Production

A 2001 survey conducted by the Harvard Center for Risk Analysis (HCRA) concluded that public risk perception and the demand for safer foods are important factors in shaping agricultural production practices, (*5*). Concern about potential health effects of synthetic pesticide residues in food and misconceptions/misrepresentations about the benefits of organic foods have been primary driving factors in consumer's willingness to pay a premium price for organic foods. Despite the potentially higher production costs and lower yields, premium prices have made it attractive for growers to switch partially or totally from conventional to organic farming.

The organic food sector is growing significantly with reported sales increasing 20 percent or more annually (*6*). Nearly half of the estimated $7.8 billion spent on organic foods in 2000 is now purchased at conventional supermarkets rather than at natural food or health stores. However, organic food purchases still represents a very small percentage in comparison to the $449 billion total that was spent during 2002 in supermarkets (*7*).

Food Safety: Risks and Perceptions

Products labeled as "natural" or "organic" are psychologically appealing. A wide variety of these products, e.g., dietary supplements, cosmetics, cleaning products, clothing, are now available. However, "natural" or naturally occurring substances are not necessarily non-toxic or risk free. For example, one cup of coffee contains about 1,000 natural substances; 30 have been tested and at least 21 of these are known to be carcinogenic to rats at high doses (*8*).

The 2001 HCRA survey found that a mix of organic and conventional food buyers in the Boston area perceived that conventionally grown food represents a more serious public health hazard than other potential risks (*5*). Perhaps these perceptions are fueld by unsubstantiated claims (e.g., positive health outcomes, superior taste, more nutritious) that may be used to promote the quality and benefits of organic products while alleging that food grown by conventional agriculture is unsafe. Exaggerated benefits and marketing based on food safety isperceptions may convince some affluent consumers to buy organic, but at the same time, low-income consumers, who can not really afford it, may also be needlessly coerced into paying higher prices for their food.

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The following list places the actual risks of dietary exposure to pesticide reisdues in the context of exposure risks to microbial pathogens and plant natural products with known toxicological properties.

- Undeniably, some foods may contain synthetic pesticide residues, but USDA analyses, conducted in conjunction with the 2002 Pesticide Data Program (PDP), indicated that approximately 57.9 percent of all the 12,899 samples tested contained no detectable pesticide residues (*10*). Of those with detectable residues, only 0.3 percent were above the already health protective and conservative food tolerances listed in the Code of Federal Regulation (CFR), Title 40, part 180 (*11*).
- Tolerances are not health standards. EPA establishes tolerances to designate the maximum pesticide residue allowable on a raw or processed agricultural commodity. Analytical methods are now sensitive enough to detect residues at parts per trillion, or less. To put this into perspective for the lay public, one part per trillion is the equivalent of one inch in 16 million miles.
- According to the American Dietetic Association, there are 76 million cases of microbial food poisonings annually in the US. Of these, 325,000 are serious enough to warrant hospitalizations that ultimately end in 5,000 deaths, (*9*). Moreover, although consumers may be concerned about potential chemical risks, because the issue usually receives more publicity, microbial pathogens and toxins (e.g., salmonella toxins in undercooked meat or ciguatera and scromboid poisoning from fish consumption) are far more serious health threats than synthetic pesticide residues.
- Both organic and conventionally grown foods contain biologically active natural compounds that have pesticide properties. These naturally occurring pesticides are produced in plants to defend against fungi, insects and other predators. On average, Americans consume 1500 milligrams of 5,000 to 10,000 natural pesticides and their breakdown products daily (*12*).
- Food and forage crops may also contain natural neurotoxins, cholinesterase inhibiting solanum glycoalkaloids (found in green potatoes and tomatoes) or photosensitizing furocoumarins (found in clover, celery, parsnips and limes) that have caused adverse effects in livestock and humans (*13*).
- Most common foods and beverages, whether grown by NOP standards or conventional agriculture, also contain substantial quantities of various, chemically diverse, naturally occurring estrogenic compounds called phytoestrogens, linked to both beneficial and harmful affects in humans and livestock (*14*). In reality, the amount of natural estrogen equivalents from the phytoestrogens in one glass of red wine far exceeds the daily intake of synthetic estrogens from organochlorine pesticide residues (*15*).

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The USDA makes no claims that organic farming is better than conventional farming (*16*). In its consumer brochure, the USDA also makes no claims that organic food is safer or more nutritious than conventionally grown food (*17*). In releasing the NOP Proposed Rule for 90-day public comment on March 7, 2000, U.S. Secretary of Agriculture Dan Glickman stated that:
"Just because something is labeled as organic does not mean it is superior, safer, or more healthy than conventional food. All foods in this country must meet the same high standards of safety regardless of their classification" (*18*).

Comparison of Organic and Conventional Farming

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Several scientifically defensible analyses have concluded that organic farming is not superior, higher yielding, or necessarily better for the environment than conventional farming.

- In comparing 21 years of organic versus conventional production for seven crops, Swiss researchers noted that organic crop yields are [10-40%] lower with a total decreased crop average yield of 20% (*19*).
- In another long-term analysis based on direct field-to-field comparisons, the Hudson Institute Center of Food Safety concluded that, depending on the crop and climate, organic yields are significantly (5–45 %) less than non-organic yields. This difference is primarily due to the lower nitrogen content of the manure fertilizers used by organic farmers as compared with synthetic fertilizers (*20*).
- In 1998 the Danish Government commissioned a study to evaluate "the overall consequences of totally restructuring of the agricultural sector for organic food production." The resultant 1999 Bichel Committee Report "Organic Scenarios for Denmark" concluded that a total shift to organic farming from conventional agriculture would result in a drastic change with considerable restrictions and not enough food production to feed the Danish population (*21*).
- A 2003 study from the National Center for Food and Agricultural Policy (NCFAP), "The Value of Herbicides in US Crop Production", reported that replacing the use of synthetic herbicides in agriculture would necessitate a significant increase, 1.2 billion hours, in hand weeding, cause a reduction in yields, from 67 to 5 percent for 35 of the 40 crops studied, and foster soil erosion because of increased tillage of the soil (*22*).

A wholesale switch to organic farming could actually result in more pesticide applications or increased environmental contamination. For example, Bt and pyrethrum extracts require more applications per season than synthetic pesticides because they are less potent and environmentally less stable. A National Center for Food and Agriculture Policy (NCFAP) summary report also revealed that in 1997 two NOP approved pesticides, crop oil and sulfur, were among the five most widely used pesticides in the U.S. (*23*). However, because sulfur and copper are more environmentally persistent than synthetic pesticides, their increased use could result in increased environmental risks. The Hudson Institute reported that in 1997 sulfur was applied at an average rate of 34.9 pound per acre, more than 22 times higher than the average use rate of 1.6 pounds per acre for synthetic fungicides (*24*). The same analysis reported that copper was used at an average rate of 4.08 pounds per acre, more than 2.5 times higher than the average synthetic fungicide use rate. Based on the Hudson Institute's figures, more than 102.8 million pounds per year of copper products would have been required to replace considerably less synthetic fungicides (40 million pounds per year).

Conventional Pesticide Development and Registration

Conventional pesticides are regulated on both the federal and state level. The Federal Insecticide and Rodenticide Act (FIFRA) and the Federal Food Drug and Cosmetic Act (FFDCA) provide the federal legislative statutory requirements. FIFRA §2(u) defines a pesticide as any substance, or mixture of substances intended for preventing, destroying, repelling or mitigating a pest (*25*). Pesticides, insecticides, rodenticides, herbicides and fungicides, and biocides, represent a wide variety of chemicals with various biological activities that are used to protect crops and the public from insects, arachnids (ticks, spiders, mites, scorpions), rodents, weeds, molds and bacteria.

Pesticide oversight also involves three federal agencies, EPA, FDA and USDA. EPA has the major role to review pesticide safety evaluations, conduct risk analyses, establish tolerances and grant registrations. The FDA is responsible for the enforcement of food tolerances. The USDA is responsible for conducting agronomic field research, essential population dietary surveys (the Continuing Survey of Food Intakes by Individuals (CSFII) used in dietary risk assessment), pesticide residue studies (the Pesticide Data Program) and providing grower education through its regional extension offices.

Synthetic pesticides are among the most extensively studied chemicals. Pesticide manufacturers are required by law to provide EPA's Office of Pesticide Programs (OPP) with sufficient data to thoroughly characterize the potential risk and to conduct a risk assessment, the process by which safe exposure levels and guidelines for proper use are established. Before approving a pesticide registration, OPP also reviews the safety of pesticide formulation ingredients and the manufacturing process (starting materials and potential impurities), the site or crop on which the pesticide is to be used (i.e., the amount, application

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practice, frequency and timing of its use), and the proposed storage and disposal practices. All the approved guidelines, warnings and restrictions must be stated on the pesticide label. The pesticide label also lists the crops and sites the product can be used on, specifies buffer zones to protect wildlife, and the direction for safe use (required personal protective equipment and limitations such as restricted entry intervals).

Pesticide registration is a dynamic process. Older pesticides are continually evaluated against new safety standards, such as the Food Quality Protection Act of 1996 (FQPA), and on-going regulatory processes - re-registration, data call-ins and tolerance reassessments. Under FIFRA §6(a)(2), "adverse effects reporting" requirements (as expounded in 40 CFR Part 159), pesticide registrants must provide EPA in a timely manner with adverse effects information, new toxicity data and any incident reports involving consumers, workers and wildlife or else face the consequences of fines (*26*). The Agency considers FIFRA §6(a) (2) adverse reports to request label changes for registered products.

Current development costs and timelines to register a new pesticide active ingredient are on average $184 million dollars and 9 years, from discovery to registration (*27*). The process to bring one successful commercial product into the marketplace generally requires screening 140,000 candidate chemicals, extensive field efficacy evaluations, and formulation development to optimize performance and ensure consumer/worker safety in handling the product.

An EPA registration data package for a food-use pesticide typically contains data from at least 120 safety evaluations (*28*) included in the following categories of testing.

- Toxicology: Multi-species acute effects of a single exposure, sub-chronic and chronic effects of intermediate and lifetime exposure, developmental and reproductive effects, mutagenic effects on genes and inherited traits, and carcinogenic effects during lifetime exposure.
- Metabolism: In both plants and animals.
- Environmental Fate: Degradation (breakdown) in soil, water, air and plants to identify potential bioaccumulation and persistent residues; movement by runoff, leaching and spray drift.
- Residues in Food and Feed: The nature and quantity of residues on raw crops, processed food, and in animal feed, meat, milk, poultry and eggs.
- Ecological Effects: Acute and chronic toxicity to birds, fish and other aquatic organisms.
- Non-Target Testing: Short and long-term effects on non-target plants, wildlife and beneficial organisms.

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Current pesticide discovery research and development focuses on identifying less toxic and environmentally safe candidates. Pesticide manufacturers also prioritize safety evaluations to identify and eliminate potentially hazardous materials. New EPA programs have expedited registration reviews for "reduced risk" and replacement products, and provided additional incentives for registrants to register alternative products. Lower use rates, using a minimum number of applications and conservative treatment-to-harvest intervals, have significantly decreased the occurrence of measurable food residues and potential risks to wildlife.

Pesticide Risk Assessment

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Before any pesticide, synthetic or NOP approved, is registered and sold in the United States, EPA evaluates both the human and ecological risk in order to ensure that the product poses no unreasonable adverse effects to humans, the environment and non-target species (*29*). EPA does not grant a pesticide label until a pesticide's risk is characterized and managed.

Risk, the probability of harm, is a function of both toxicity (hazard) and exposure (eq. 1).

$$\text{Risk} = f\,(\text{toxicity, exposure}) \qquad (1)$$

Potency, dose, and dose-response, critical elements in evaluating the toxicity of all chemical substances are only partial requirements in assessing the risk of synthetic pesticides and NOP approved products. EPA also evaluates all the potential sources of relevant exposure associated with the use of a particular product.

In assessing dietary risk assessment, EPA establishes a daily exposure limit, the Reference dose (RfD) based on the No Observable Adverse Effect Level (NOAEL), the most sensitive endpoint (an exhibited toxicological effect) from among the more than 20 toxicology tests required for pesticide registration (*30*). The NOAEL is expressed in milligrams per kilograms per day (mg/kg/d). The RfD (also expressed in mg/kg/d) is derived from the NOAEL by the application of safety factors to account for inter-species variability (10x: for differences from extrapolating animal to human testing) and for interspecies variability (10x: for differences among humans, including sensitive subpopulations) (eq. 2).

$$\text{Reference Dose (RfD)} = \text{NOAEL}/(10 \times 10) \qquad (2)$$

FQPA mandates use of the FDA standard, "reasonable certainty of no harm," in regulating pesticides (*31*) and special consideration of children and other sensitive subpopulations in conducting risk assessments. For characterizing dietary pesticide risk, the FDA standard is represented as a Population Adjusted Dose (PAD), an exposure maximum derived from the RfD that is divided by additional FQPA safety factors (up to 10x) if there is concern about pre- and post-natal toxicity or if the database for exposure and toxicity to infants and children is incomplete (*32*) (eq. 3).

Population Adjusted Dose (PAD) = RfD/FQPA Safety Factor (1, 3, or 10x) (3)

PADs are calculated to consider one day of exposure (acute) and daily exposure over a 70-year life span (chronic). The acute and chronic PADs are expressed as mg/kg/day.

FQPA has also required EPA to evaluate potential acute exposures and multiple pathways of acute and chronic exposures - dietary, drinking water and residential exposures - in assessing risk for single chemicals (aggregate risk) and for multiple chemicals with a common mechanism of toxicity (cumulative risk).

Pesticide dietary exposure (eq. 4) is derived by multiplying the amount of a pesticide residue that is present in and on a food (the residues associated with the raw and processed agricultural commodity) and consumption (the percentage types and amounts of food consumed by various populations, differentiated by age groups, e.g., infants, children and adults).

Dietary Exposure = Consumption x Residue (4)

The population dietary consumption information is derived from the USDA CSFII survey data. Pesticide manufacturers conduct multiple field trials under various climatic conditions, at maximum use rates and number of field applications, to generate a "worse to best case" residue profile. Taken collectively, the dietary exposures (the maximum residues of all the raw and processed foods) from all of the registered crop uses cannot exceed 100% of the PADs.

NOP Product Safety, Registration and Risks

Some NOP approved products are pesticides subject to the same legislation (FIFRA, FFDCA) and EPA regulations and enforcements as the pesticides used in conventional farming. The manufacturers and registrants of NOP products are also legally bound by the adverse effects reporting requirements of FIFRA §6(a) (2).

Some of the NOP products are naturally occurring botanical pesticides, but NOP approved products are rarely used as they occur in nature, and like

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synthetic pesticides, must undergo manufacturing that includes purification procedures. For example, pyrethrums are extracted from plants and flowers of *Chrysanthemum cinerariafolium*, and the lead which is often a contaminant of elemental sulfur must be removed. NOP pesticides must also be formulated with inert ingredients to enhance product efficacy, facilitate application and improve environmental stability.

Crops produced using NOP-approved products must also meet established limits for naturally occurring neurotoxic and carcinogenic food toxins. As with conventional agriculture, precautions must be taken to avoid the proliferation of harmful fungi and bacteria that may generate dangerous poisons such as aflatoxin, ergot alkaloids, salmonella and botulinum toxin.

An increase in commercial organic food production could lead to an increase in commercial and home garden use and potential exposure to NOP approved pesticides. Because of a long history of use, some of the NOP approved chemicals have been exempted from the tolerance setting process and thus have no legal limits for residues on food. However, lacking the analyses to detect residues does not necessarily mean that there are no NOP approved pesticides or harmful microbes (such as salmonella) on or in organic foods.

The public should be aware that if not used safely and judiciously, all chemical substances, including the NOP approved products, have the capacity to cause harm to the environment and to people. Paracelsus (1493-1541), the "father of toxicology", is credited with saying "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy". This statement is also commonly paraphrased as "the dose makes the poison," or alternatively, "there is a safe level of everything."

Consumers and workers who may come in contact with NOP products should be informed about potential worker safety issues.

- Copper sulfate, widely used as a fungicide in US vineyards, is persistent in the soil and toxic to aquatic organisms and earthworms. The European Union (EU) banned the use of copper sulfate in 2002 because of liver toxicity to vineyard workers (*33*).
- Pyrethrum extracts may cause allergic skin and asthma reactions in sensitive individuals (*34*).
- *Bacillus thuringiensis* (Bt) spores are reported to cause fatal lung infections in mice (*33*).

Detailed health and environmental risk information for some NOP approved pesticides, including sulfur (*35*), rotenone (*36*) and boric acid (*37*), can be found in EPA Re-registration Eligibility Decision (RED) Fact Sheets.

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Sulfur

Elemental sulfur has been used as an insecticide, fungicide and rodenticide on several hundred food and feed crops, turf and ornamentals, and for other residential applications since the 1920's. The public is generally exposed to sulfur through food residues. Extensive use as a fungicide in vineyards by organic and conventional farmers has resulted in contact (eye irritation) and dermal toxicity worker exposure issues. As a result, EPA recommends protective clothing and a 24-h reentry interval for farm workers after foliar applications of sulfur. Furthermore, epidemiology studies on mine workers exposed to sulfur dust and sulfur dioxide throughout their lives revealed the occurrence of eye and respiratory disturbances, chronic bronchitis, and chronic sinus effects.

Rotenone

Rotenone, a botanical insecticide derived from the root of various tropical plants, is registered for a variety of commercial and home garden uses. Rotenone is highly toxic to fish and causes Parkinson-like symptoms in rodents (*38*). The Merck Index also states that rotenone may cause severe pulmonary edema and over-exposure could result in irritation of eyes, skin, and respiratory systems; numbness of mucous membranes; nausea; vomiting; abdominal pain; muscle tremors; incontinence; and clonic convulsions (*39*). EPA currently lists the active ingredient as Category III toxicity but formulation as an emulsifiable concentrate substantially increases the toxicity to Category I.

Boric Acid

Boric acid also has a long use as an insecticide, fungicide and herbicide and current EPA records show 126 active product registrations. Boric acid and its derivatives are also recommended for NOP structural pest control, but treatment should avoid any direct contact with organic foods. Boric acid is not reported to be mutagenic or carcinogenic but rodent and non-rodent chronic studies have demonstrated testicular effects and decreased body weight at high doses. Developmental and reproductive studies revealed maternal liver and kidney effects and decreased body weight in the dams and the pups. Formulated boric acid can be used quite safely as an effective method for controlling cockroaches. However, if left in an open dish boric acid powder may present a greater threat to children and pets than indicated by the acute laboratory animal testing data. Although the Merck Index reports a relatively low rodent toxicity (rat LD50 of 5

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g/kg), human deaths have been reported at much lower doses. Ingestion of less than 5 grams has caused death in infants and amounts of 5 to 120 grams have caused death in adults (*40*). The Merck Index also reports that ingestion or absorption of boric acid may cause nausea, vomiting, diarrhea, abdominal cramps, erythematous lesions on skin and mucous membranes, circulatory collapse, tachycardia, cyanosis, delirium, convulsions, and coma.

Conclusions

The USDA does not advocate that organic food is safer or more nutritious than conventionally grown food. Therefore, the proponents of organic farming and foods should not mislead the public about benefits or discredit products derived from conventional agriculture.

The USDA NOP is necessary to provide consistent standards and marketing guidance for organic farmers as well as federal and state regulators. Certified organic products are not pesticide free. Some NOP-approved products are registered pesticides. Consumers should be aware of potential safety issues and properties of the NOP-approved products that are used in growing USDA certified organic produce. However, FIFRA regulations apply to all pesticides, regardless of farming system, and are designed to ensure that health and environmental safety are thoroughly investigated by EPA before a product enters the marketplace.

The safe and judicious use of all pesticides benefits society and agriculture. USDA PDP analyses continue to demonstrate that the U.S. food supply meets the FDA standard of "reasonable certainty of no harm", therefore deomonstrating its safety (10). At the August 2002 press briefing announcing FQPA cumulative risk assessment for the organophosphorus insecticides (*41*), Stephen Johnson, then acting EPA Assistant Administrator, stated, "The rigorous scientific and public process followed by EPA during the [FQPA] tolerance reassessment continues to strengthen our confidence in the overall safety of the nation's food supply."

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Chapter 7

A Reduced Risk Insecticide for Organic Agriculture: Spinosad Case Study

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Spinosad is a naturally-derived, insecticide generated during fermentation by the actinomycete bacteria *Saccharopolyspora spinosa*. Spinosad was approved for use in the U.S. on cotton and turfgrass during 1997 as part of EPA's reduced risk pesticide program based on its low mammalian toxicity, low environmental impacts, and compatibility with integrated pest management. As of 2005, spinosad has been approved for use on more than 150 fruit and vegetable crops in the U.S. and also in more than 70 other countries. Due to its unique, natural products origin and fermentation-based manufacturing, spinosad has been approved for use in certified organic agriculture in the U.S. by the USDA National Organic Standards Board. Use of spinosad products in organic agriculture has also been authorized by other government and private certifying bodies in the U.S. including the Organic Materials Review Institute, and by similar organizations in other countries including Argentina, Australia, Guatemala, New Zealand, Peru, and Switzerland. This chapter provides a review of spinosad development, registration, and manufacturing efforts with particular attention to the approval and use of spinosad products in organic agriculture.

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
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Agrochemical discovery efforts are grueling and exhaustive searches for the proverbial "needle in a haystack". For every product that enters development, more than 100,000 candidate compounds and analogues will have been screened at one level or another (*1*). The discovery, characterization, and development phases are collectively both costly and time-consuming. It takes on average approximately 9 years to move from an exciting discovery in the laboratory to commercialization and an average investment of more than $150 million (*1*).

Discovery and Characterization

Dow AgroSciences (formerly DowElanco) was formed during the 1980's as a joint venture of the Dow Chemical Company and Eli Lilly and Company (Dow later assumed full ownership), and both organizations brought a strong emphasis on evaluation of natural products as well as synthetic ones as potential pesticidal products. Thus, Dow AgroSciences interest has included evaluation of fermentation broths of soil microorganisms, live microorganisms, plant extracts, marine organism extracts, and insect toxins.

Screening efforts during the mid-1980's identified insecticidal activity in a fermentation broth isolated from a soil sample collected several years earlier (Table I). An initial screen demonstrated activity in a mosquito larval assay (*Aedes aegpyti*), and this activity was confirmed in a subsequent larval Lepidoptera assay (*Spodoptera eridania*) (*2*). Soon thereafter, the insecticidal activity was attributed to natural fermentation metabolites generated by a newly discovery soil bacterium (Order Actinomycetales, fungus-like bacteria), which was named *Saccharopolyspora spinosa* (*3*).

The natural metabolites responsible for the insecticidal activity were termed "spinosyns". Subsequent work determined the chemical structure of the spinosyns as a suite of structurally related macrolides (*4*). The spinosyn molecule is built around a unique tetracyclic ring system to which two different sugars are attached. The most prominent and active of these compounds were spinosyn A and spinosyn D, and collectively these have been designated as the active ingredient "spinosad" (Fig 1). Spinosad active ingredient typically contains spinosyns A and D in roughly a 5:1 to 6:1 ratio.

Additional efficacy testing revealed that spinosad demonstrated excellent insecticidal activity against a broad spectrum of pest Lepidoptera and Thysanoptera. In addition, spinosad was found to be highly active against other insects including selected Diptera, Coleoptera, Orthoptera, Siphonaptera, and Anoplura (*5*). Spinosad was also determined to have a unique mode of insecticidal action, distinct from all other known insecticides (*6*).

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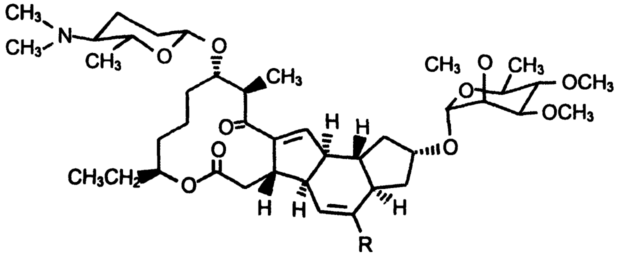

Figure 1. Chemical Structure of Spinosad

Table I. Milestones in Spinosad Discovery, Development and Registration

Date	*Milestone*
1982	Soil sample collected from rum distillery in the Virgin Islands
1985	Screening of fermentation broth from soil sample demonstrates biological activity toward mosquito and southern armyworm larvae
1985	Newly discovered bacterium isolated from fermentation broth, *Saccharopolyspora spinosa*, found to produce active substances named "spinosyns"
1988	First field efficacy trials initiated
1989	Structure of spinosyn A determined
1991	Predevelopment regulatory research program initiated
1994	Product commercialization and development program initiated
1995	Submission of full registration data package to U.S. EPA and other regulatory authorities
1996	First global registration approval of spinosad in Korea
1997	First U.S. registration approval for cotton, turfgrass and ornamentals
1999	Spinosad recognized with Presidential Green Chemistry Challenge Award for "designing safer chemicals"
1999-2001	First organic agriculture approvals by bodies in Switzerland, Tunisia, and U.S.
2002	USDA National Organic Standards Board approval of spinosad for use in certified organic agriculture
2003	First set of Codex maximum residue limits (MRL's) established for spinosad following WHO and FAO evaluations

Development and Registration Testing

Following characterization of the insecticidal activity of spinosad, a key decision regarding pursuit of a commercial development program had to be reached. Given the time and cost involved in the development and registration process, the decision to proceed with a candidate such as spinosad was a significant one. Early biological efficacy and safety testing identified spinosad as a highly efficacious and low human/environmental impact compound. The decision to proceed forward with testing that would lead to commercialization was reached during 1990.

In light of the extremely high activity shown by spinosad against key pest Lepidoptera, initial development efforts were focused on agricultural use on cotton and non-agricultural use on turfgrass and ornamental plants. Biological testing efforts were quickly focused on two different soluble concentrate (SC) formulations, Tracer* (44.2% active ingredient) for agricultural use and Conserve* (11.6% active ingredient) for non-agricultural use (*Trademark of Dow AgroSciences). Small-plot field research trials on cotton through 1994 were focused on the Heliothine pests and beet armyworm (*Spodoptera exigua*), with research conducted in the U.S. as well as Australia, Brazil, Colombia, Egypt, Greece, India, and Pakistan. Beginning in 1995, large-plot (~10 acres) cotton field trials were initiated in the U.S. through an extensive experimental use permit (EUP) program (*7*). A comparable turf and ornamental field EUP program was initiated in the U.S. during 1996, and it was focused on pest cutworms (e.g., *Agrotis ipsilon*), armyworms (e.g., *Spodoptera frugiperda)*, and webworms (*Parapediasia teterella*) (*8*). Promising results from both programs confirmed the effectiveness of spinosad at low use rates ranging from 0.045-0.123 kg/ha (0.04 to 0.11 lb ai/acre) (*7,8*).

An intensive predevelopment registration testing program of chemistry, toxicology, and environmental studies was initiated for spinosad during 1991. In addition to laboratory studies with various test organisms and systems, field studies to characterize the behavior of spinosad residues in soil, water, and on plants were also conducted. Following completion of the core registration data package during 1995, it became clear that spinosad possessed an extremely favorable combination of properties from a registration standpoint with respect to both human and environmental safety considerations (Table II). Thus, it qualified for a reduced risk classification and accelerated regulatory evaluation at U.S. EPA based on its lower mammalian toxicity, lower environmental impacts, and greater compatibility with IPM programs as compared with available alternatives (*9*).

It is important to emphasize the significant database of Dow AgroSciences and independent testing information that undergirds the efficacious use of spinosad. In addition to the core registration and efficacy testing data required for evaluation by government authorities and universities, researchers have

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Table II. Key Regulatory Properties of Spinosad

Reduced Risk Criteria	*Properties*
Low mammalian toxicity	Rat (oral) LD50 >5000 (F) and >3738 mg/kg (M) Rabbit (dermal) LD50 >2000 mg/kg Very slight dermal and ocular irritant Not a skin sensitizer Not mutagenic or carcinogenic
Low environmental impacts	Soil half-life = 9 – 17 days Soil sorption Kd = 5 - 323 (low mobility) Daphnid LC50 = 14 mg/L; Trout = 30 mg/L Quail LD50 > 1333 mg/kg
Compatibility with IPM	Low toxicity to beneficial predators/parasitoids Unique mode of insecticidal action

Sources: (*9,10*)

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actively communicated this storehouse of information on spinosad in the peer-reviewed scientific literature. Key publications for spinosad are available concerning its discovery and characterization (*2-5*), biological efficacy (*11,12*), residue chemistry and analysis (*13-15*), mammalian toxicology (*16-19*), environmental fate and impacts (*20-22*), and safety to beneficial insects and arthropods (*23,24*).

Registration History

The distinction for the first registration approval for spinosad goes to South Korea, which approved use of the product on vegetables during 1996. The U.S. registration for use of spinosad on cotton as well on turf and ornamentals occurred during February, 1997. Spinosad was the first food-use new active ingredient approved by EPA following implementation of the Food Quality Protection Act, which imposed stringent new evaluation criteria concerning human exposure and risk assessment. U.S. approvals for fruiting vegetables, brassica vegetables, leafy vegetables, apples, and citrus followed during April, 1998. Since that time, there have been a significant number of label expansions for a large number of fruit, vegetable, and nut crops, and as of 2004 spinosad has been approved for use on more than 150 crops. A critical partner in the rapid label expansion of spinosad uses has been the USDA IR-4 (Interregional Research Project No. 4) Center for Minor Crop Pest Management. The IR-4 program has been responsible for collaborating on obtaining data and petitioning

EPA in support of a large number of minor crops ranging from asparagus to cranberry to mint. USDA has also been an important partner in the current expansion of spinosad use for stored grain pest control, for which registration approval was granted during 2005.

In addition to crop uses, spinosad has also been approved by U.S. EPA for fire ant control and for housefly control in and around livestock facilities, both uses that involve bait formulations. Through a partnership with Eli Lilly and Company, spinosad has also been developed for animal health uses, and U.S. approval for an external cattle spray/pour-on product to control ectoparasites occurred during 2002.

Although most spinosad registrations around the world have involved traditional SC formulations or, in a few instances granular bait products (Table III), the GF-120 fruit fly bait has been a unique offering approved in the U.S. during 2002. This dilute (0.02% a.i.) liquid bait product was developed jointly by Dow AgroSciences and the USDA-ARS Fruit Quality and Fruit Insects Research Unit under a cooperative research and development agreement. Plant proteins and sugars that are highly attractive and phagostimulating to many tephritid fruit fly species comprise the bulk of the bait. After dilution, GF-120 is typically applied to susceptible fruit crops such as citrus, apples, pears, peaches, and olives as well as other crops and non-crop vegetation to control fruit fly outbreaks aerially by ULV spray or by targeted ground sprays at very low use rates of 0.0019-0.00038 kg ai/ha (0.00017-0.00034 lb ai/acre). In the U.S., EPA has granted a blanket tolerance of 0.02 ppm on all raw agricultural commodities to allow the widespread use of this bait product. This fruit fly bait has been an important tool in combating fruit fly infestations, often on an emergency basis, in California, Florida, and Central America.

Spinosad also received one the nation's top environmental honors, the Presidential Green Chemistry Challenge Award, during 1999. This award recognized technologies that incorporate the principles of green chemistry into chemical design, manufacture, and use, and spinosad was honored as a new, natural product for insect control with environmentally compatible characteristics.

On an international basis, as of 2004 spinosad had been approved for use in more than 70 countries including Australia, Brazil, Canada, Italy, Japan, Mexico, and UK. Addition of spinosad to the EU-wide Annex I listing of approved active ingredients was also nearing finalization. Spinosad has been classified by the WHO (World Health Organization) International Programme on Chemical Safety as a product "unlikely to present acute hazard", which represents the most favorable of 5 classifications recognized by this advisory body. The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) completed a comprehensive evaluation of spinosad toxicology, plant and animal metabolism, and residue chemistry during 2001, and based on the positive outcome MRL's to support international trade were promulgated by the Codex

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Table III. Spinosad Formulated Products

Type[a]	*Active Ingredient Conc (w/w)*	*Trade Names*	*Primary Uses*
SC	44.2%	Tracer[b] Audienz[b]	Agriculture
SC	22.8%	Success[b] Spintor[b]	Agriculture
SC	11.6%	Conserve[b]	Agriculture Turf and ornamental
SC	0.5%	Spinosad 0.5% SC	Home and garden U.S. Organic: List 4 inerts
WP	80.0%	Entrust[b]	Agriculture U.S. Organic: List 4 inerts
SC	0.02%	GF-120 Fruit Fly Bait[b] Success 0.02 CB[b]	Fruit fly baiting U.S. Organic: List 4 inerts
GR	0.015%	Justice Fire Ant Bait[b] Conserve Fire Ant Bait[b]	Fire ant baiting U.S. Organic: List 4 inerts
GR	1%	Biospin[b] Iprasan[b]	Housefly baiting
EC	2.5%	Extinosad[c]	External livestock pour-on and spray

[a] SC = suspension concentrate, WP = wettable powder, GR = granular, EC = emulsifiable concentrate

[b] Trademark of Dow AgroSciences

[c] Trademark of Eli Lilly and Company

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Alimentarius Commission during 2003. The WHO Pesticide Evaluation Scheme (WHOPES) was also currently evaluating spinosad as a future candidate for mosquito larvae control.

Fermentation Source

As a basic producer of crop protection chemicals, Dow AgroSciences has a strong history of chemical synthesis and manufacturing. In conjunction with the manufacturing of spinosad, a world-class capability in fermentation technology has also been developed. The natural fermentation origins of spinosad have been continued in commercial manufacturing. Unlike some other product classes for which natural origins later gave rise to synthetic analogues (e.g., natural pyrethrins from *Chrysanthemum* as the forerunners of synthetic pyrethroids), commercial production of spinosad today still involves the labors of the same, humble soil bacterium first isolated in the early 1980's.

Microbiology

Saccharopolyspora spinosa is the soil bacterium that during 1985 was discovered to produce spinosad (3). The sample from which this microorganism was first isolated was collected from soil inside a defunct sugar mill rum still in the Virgin Islands. *S. spinosa* is a gram-positive, non-motile, spore-forming, filamentous bacterium or actinomycete. The Genus *Saccharopolyspora* was previously established based on the type species *S. hirsute*, which was isolated from sugarcane bagasse. The assignment of the newly discovered, spinosad-producing bacterium to this Genus was based on a suite of observed cultural, morphological, and physiological characteristics; the species name *spinosa* was based on the very distinctive spiny exterior surface of the bacterial cells observed under microscopic magnification. This species name also formed the basis for the nomenclature of spinosad and the spinosyns.

During growth and aerobic fermentation activity, *S. spinosa* produces as secondary metabolites the spinosyns that comprise spinosad. The proposed biosynthetic pathway involved is thought to comprise three primary series of steps. First, the core macrolide structure appears to be formed by successive addition of nine acetate and two proprionate units. Second, the rhamnose sugar unit is formed and bound to the macrolide core. Third, the forosamine sugar unit is synthesized and bound to the macrolide core. The genetic basis for the biosynthesis has also been investigated (*25*).

The fermentation culture conditions under which *S. spinosa* produces spinosad requires aeration to maintain oxygenated conditions. A favorable aqueous growth medium contains proteins, carbohydrates, oils, and minerals (e.g., corn solids, cottonseed flour, soybean flour, glucose, methyl oleate, calcium carbonate) (*26*).

Manufacturing

Fermentation manufacturing of spinosad occurs at the Dow AgroSciences facility located in Harbor Beach, Michigan using patented processes (*26*). Effective deployment of the spinosyn synthetic pathway is the responsibility of *S. spinosa*, and the role of this state-of-the-art fermentation facility is to create the correct conditions under which this fascinating microbe can do its duty.

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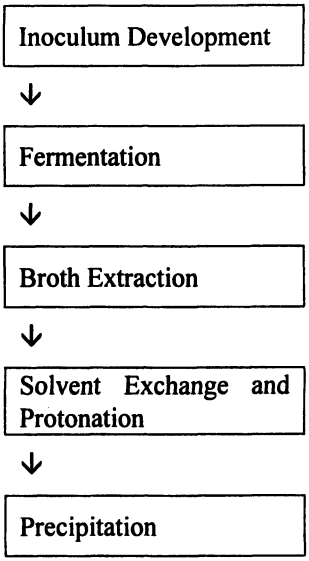

Figure 2. Spinosad Manufacturing Steps

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There are 5 major steps involved in the fermentation manufacturing of spinosad (Figure 2).

First, an inoculum of *S. spinosa* is developed at sufficient scale to serve as a seed for an upcoming fermentation run. Although strain development efforts on the part of Dow AgroSciences microbiologists have identified mutants for production with increased spinosad-producing capabilities, no genetic engineering techniques are employed in the strain improvement process and no genetically modified organisms are used for manufacturing.

Second, a large-scale fermentation is completed. Each fermentation cycle begins with inoculation of a fresh batch of sterile growth medium. Vegetative inoculum is grown by a submerged aerobic fermentation process. The aqueous growth media contain proteins, carbohydrates, oils, and minerals. Corn solids, cottonseed flour, soybean flour, glucose, methyl oleate, and calcium carbonate may be part of the media. During the period of fermentation, spinosad accumulates in the fermentation broth.

Third, at the end of the fermentation period the accumulated spinosad is extracted from the spent fermentation broth by a solvent such as methanol. The solvent solution is centrifuged or filtered to remove solids and then is concentrated by distillation.

Fourth, spinosad present in the concentrated extraction solvent is back-extracted into an acidified aqueous solution.

Fifth, the spinosad in the aqueous solution is precipitated following base neutralization. Crystals of spinosad are then de-watered and dried for use in formulated products. The technical spinosad product typically contains about 90% spinosyns and 10% impurities from the growth medium.

Certified Organic Approvals

Early Experiences

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In light of the natural fermentation origin of spinosad and its basic nature as a biopesticide, interest arose early on the part of growers with respect to the use of spinosad in organic agriculture. From a commercial standpoint, the first limited launch of spinosad for cotton occurred during 1997, and full launch on cotton and other crops occurred during 1998. In the U.S., the first recognition of the potential utility of spinosad for organic agriculture came at the state level. For example, the Colorado Department of Agriculture added SpinTor SC to its listing of approved pesticides for use on certified organic farms during 1999. Similarly, the Texas Department of Agriculture authorized temporary approval for use of Tracer SC in organic cotton. Outside the U.S., local listings for use of spinosad products were recognized in Switzerland by FIBL (Audienz), in Tunisia by the Ministry of Agriculture (Tracer), and in Austria by AustriaBioGarantie (Iprasan). In most cases these authorizations or recommendations resulted from local, grassroots requests on the part of growers to the organic listing/certifying body.

Early experiences with other organic listing/certification bodies, however, raised some questions and concerns about the ease with which spinosad would be adopted for use in organic agriculture. Basic concerns also surfaced concerning the inherent nature of the divergent organic product evaluation and approval processes employed. For example, during 1988 the Organic Materials Review Institute (OMRI) of Eugene, Oregon, an influential certification body in the U.S. and overseas, determined that the active ingredient spinosad was determined to be non-synthetic and therefore allowed for organic production. However, OMRI did not agree to list the formulated spinosad-containing product under consideration, Success SC, because one or more co-formulants/inerts were "unresolved" as to their organic suitability. Likewise, the Bio-Dynamic Institute (IBD) in Brazil rejected use of Tracer SC for organic agriculture due, not to the active ingredient composition, but rather the non-active ingredient, co-formulant content. How could spinosad be acceptable for organic growers in Colorado and Texas and Switzerland, but not for organic growers in Brazil or those in the U.S. with allegiance to OMRI? Since the mixed experience of spinosad with certifying/listing bodies occurred also at a time when available market research was showing a rapidly expanding but rather uncertain commercial value, some reevaluation and redirection in organic certification efforts for spinosad was in order. However, some harmonization of organic recognition processes also appeared necessary for further progress especially in the U.S., since it had become all too apparent that rather than a unified, monolithic organic certification/listing process what instead existed was a crazy-quilt of government and private, local and national authorizing bodies with disparate criteria and definitions.

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U.S. Organic Agriculture

A couple of major developments during the late 1990's paved the way for a much clearer definition of the approval process for organic agriculture inputs in the U.S. The first of these developments involved USDA. Although the 1990 Organic Food Production Act (OFPA) established the basis for a national organic program, it wasn't until December, 2000 that final standards for operation of the USDA National Organic Program (NOP) were promulgated (*27*). Thus, a well-defined evaluation process for creating a single, nationally recognized list of approved substances for use in organic agriculture was created. In addition, USDA established criteria with respect to co-formulants, by recognizing that any substance on the national list was suitable for organic agriculture only if any co-formulants were classified by U.S. EPA as List 4 inert ingredients.

The second development, then, involved U.S. EPA and product labeling criteria. During early 2003, U.S. EPA published its long-awaited policy with respect to labeling of pesticide products under the National Organic Program, which authorized the addition of the statement "For Organic Production" to the label of those products containing 1) active ingredients on the USDA NOP National Listing and 2) containing only EPA List 4 inert ingredients (*28*).

A petition to request evaluation of spinosad as a NOP listed active ingredient was submitted to the USDA National Organic Standards Board (NOSB) during early 2002. In advance of the evaluation meeting, it is interesting to note that dozens of letters to support a positive listing of spinosad poured into USDA on behalf of various state and federal programs, universities, crop consultants, grower organizations, and even foreign governments (especially Central America where the MOSCAMED fruit fly eradication program was in operation). During May, 2002 the USDA NOSB completed its evaluation of spinosad and determined that, due to its natural, fermentation source and relatively benign toxicological profile, spinosad was compatible with organic agriculture and allowed for use in organic agriculture. This was a major milestone, then, in the recognition of spinosad for U.S. organic agriculture.

With respect to formulations most appropriate for use in organic agriculture and containing only EPA List 4 inert ingredients, Dow AgroSciences narrowed its initial focus in the U.S. to 4 primary products (Table III). This was not an easy task since some commonly employed co-formulants (e.g., surfactants, emulsifiers, stabilizers), especially those for liquid formulations (e.g., SC, EC), are not on List 4 but rather appear on other EPA approved inert lists (e.g., List 3). The first product of focus was a wettable powder for agricultural use. Entrust 80 WP was a slightly modified version of a wettable granule that had been developed and registered earlier but had not been previously commercialized. The second product was an existing granular, corn-grit based bait intended for fire ant control first developed and registered during 1998 (Conserve, Justice). The third product was a liquid concentrate for dilution and spraying as a fruit fly bait (GF-120), for which a slightly modified version (GF-120NF) of a product first approved for emergency use during 2000 was developed. This slight modification involved removal of a synthetic preservative. Labels for these formulated products, containing only spinosad and List 4 inert ingredients, were approved by U.S. EPA during 2003 following promulgation of the EPA organic labeling policy. A more recently developed product is Spinosad 0.5% SC, which is available for home and garden use.

Organic certifications and listings for spinosad products continue to occur in the U.S. by state agencies and private certifiers. For example, the Washington Department of Agriculture approved Entrust for use in organic food production during 2003. Also, the Organic Materials Review Institute (OMRI) evaluated spinosad formulated products and decided during early 2003 to approve the use of Entrust, GF-120NF, and Converve Fire Ant Bait for organic agriculture (Table IV).

Although the introduction and adoption of spinosad products in the U.S. for organic agriculture is still at an early stage, there have been some early successes. Of particular note was the utility of spinosad in addressing a crisis which developed in California during 2002, when a significant agricultural segment of the state was threatened by infestation of the Mexican Fruit Fly. The California Department of Food and Agriculture (CDFA) approved the emergency use of GF-120 NF within a 28-mile quarantine section of San Diego County on crops including grapefruit and avocado. Since there were a significant number of organic growers present in the quarantine zone, the organic certification status of spinosad and the GF-120 NF product was a critical component to the success of this program.

International Organic Agriculture

Outside the U.S., there are a variety of government and private certifiers of organic agriculture and allowable inputs, including pesticides, and there may be

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Table IV. Examples of Spinosad Organic Approvals

Country	*Certifying Body*	*Product(s)*
Argentina	Senesa Organics	Entrust
Australia	Australia Certified Organic Pty Ltd	Entrust 80W Naturalyte Fruit Fly Bait
Guatemala	Mayacert BCS Oko-Garantie	GF-120NF
New Zealand	Bio-Grow	Entrust 80W
Peru	Senasa	Success 0.02 CB
Spain	Sociedad Espanola de Agricultura Ecologica (SEAE)	SpinTor 48 SpinTor CEBO (GF-120)
Switzerland	Forschungsinstitut fur biologischen Landau (FIBL)	Audienz SC
Tunisia	Ministry of Agriculture	Tracer SC
U.S.	USDA National Organic Standards Board	Technical Spinosad
U.S.	U.S. EPA Office of Pesticide Programs	GF-120 NF Entrust 80W Conserve Fire Ant Bait Spinosad 0.5% SC
U.S.	Colorado Department of Agriculture	SpinTor
U.S.	Washington State Department of Agriculture	Entrust GF-120 NF
U.S.	Organic Materials Review Institute (OMRI)	GF-120 NF Entrust 80W Conserve Fire Ant Bait Spinosad 0.5% SC

a single body or multiple approval/listing bodies within each country. Although growers in some countries reference certifying agencies or bodies in their own countries, in other cases growers or grower organizations reference and/or are certified by foreign groups. In fact, it is common for growers in agricultural exporting countries to be required to have certification by bodies in the countries to which they ship their organic produce. For example, organic coffee growers

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in Central America export mostly to the U.S. and Europe, so reference to the USDA NOP listing of approved products or certification by authorizing bodies in some European countries (e.g., BCS Oko-Garantie in Germany) may be required. Similarly, organic fruit or flower growers in Africa may need to secure certification and use only pesticides approved by such European bodies as Ecocert or MPS in order to ship organic products to Germany or the Netherlands, respectively.

Spinosad products have been approved/listed for use in organic agriculture by a number of organizations outside the U.S. (Table IV). Early approvals came in Switzerland and Tunisia. More recent approvals during 2003 have come for specific products in Argentina, Australia, Guatemala, New Zealand, and Peru. In some countries (e.g., Australia, New Zealand) the specific formulations developed and approved for use in the U.S., particularly Entrust and GF-120NF, have been the products also compatible with national organic product requirements. In other countries, organic product requirements are primarily focused on the active ingredient rather than the co-formulants. For example, in the European Union, a great degree of scrutiny is placed on products of microbial origin to ensure that no involvement of genetically modified organisms or their byproducts are utilized.

As far as international standards for organic agriculture, the Codex Alimentarius Commission has developed guidelines for the production, processing, labeling, and marketing of organically produced foods (*29*). Ongoing activities through the Joint FAO/WHO Food Standards Program and the Codex Committee on Food Labeling continue to grapple with considerations related to development of an international listing of pesticide products suitable for use in organic agriculture, but competing national priorities and agendas have thus far interfered with significant progress at the international level.

The greatest single success for spinosad with respect to organic agriculture outside the U.S. has been associated with the MOSCAMED program in Central America (*30*). This cooperative program of the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) and the governments of Mexico and Guatemala is targeted at preventing the invasion of North and Central America by the Mediterranean Fruit Fly or Medfly. So far, control efforts have focused on creation of a Medfly-free zone across the Central American isthmus and have included area-wide application of pest suppression practices. The spinosad fruit fly bait GF-120NF has been a primary tool of the MOSCAMED program due to both its highly efficacious nature and favorable environmental profile when considered for area-wide, low-volume spraying. In addition, the organic certification of spinosad by USDA and of GF-120NF by regional organic certifiers such as Mayacert, have enabled the use of the product across a regional landscape shared by both conventional and organic growers.

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Benefits and Future Considerations

The recognition of spinosad as suitable for use in certified organic agriculture has made a highly efficacious product with a highly favorable regulatory profile available to organic growers both in the U.S. and around the world. In addition to individual grower benefits, spinosad has also proven to be a critical component of area-wide control programs (e.g., Medfly) from an efficacy and public acceptability standpoint, most particularly when the area of infestation has included both conventional and organic farms and orchards. For management of the pests for which spinosad is effective, there is no longer a need for growers with organic interests or inclinations to choose between certified products of often dubious performance and effective products of synthetic chemical origin.

Involvement in organic certification efforts for spinosad has also been a key learning experience for a basic agricultural chemical producer such as Dow AgroSciences with respect to the needs and requirements of organic agriculture. This experience has included exposure to both the positive aspects and foibles of the organic movement. Particularly eye-opening has been the extremely high focus on origin and composition of pest management products which organic growers are willing to employ, with human and environmental safety factors important but clearly secondary considerations. For example, the basic tenets of organic agriculture would seem often to favor naturally-derived products which may in some cases lack the comprehensive safety testing (e.g., chronic mammalian toxicity, environmental chemistry, residue chemistry) and risk assessments required for synthetic chemicals. Hopefully, spinosad will be only one of a number of modern and well-tested products of natural origin available to the organic grower.

Future Dow AgroSciences efforts may be focused on both expansion of uses for existing organic formulations and development of additional organic formulations for various organic agriculture market segments. For example, U.S. approval of spinosad for stored grain use included submission of both conventional SC and organic (Entrust 80W) formulations. In addition, efforts at obtaining recognition of spinosad's utility in organic agriculture in other countries will be continued. Most notably this will include additional efforts in EU member states following the Annex I listing of spinosad as an approved active substance. Finally, interest in advancement of other products for use in organic agriculture will also be continued.

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Chapter 8

Environmental and Health Assessments for Spinosad against the Backdrop of Organic Certification

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Spinosad is often highlighted as a product that combines the best characteristics of the biological insecticides and the synthetic insecticides. As such spinosad has recently been certified as organic for crop and livestock uses based on its natural origin and the fact that it is produced via fermentation. This chapter focuses on the environmental and health safety aspects of spinosad against the backdrop of organic certification. Web searches of public registration information provide the canvas for comparison of spinosad to several of its organic counterparts. Specifically, there is robust documentation of spinosad's favorable safety profile with low mammalian toxicity, low toxicity to most non-target organisms and rapid degradation in several environmental matrices. In addition, a key aspect of managing this popular active ingredient has been refinement of estimated dietary intake to allow for continued development and introduction of new uses.

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
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Why Spinosad?

Spinosad is not just another insecticide. It is emerging as an important alternative to older classes of insecticidal products. Spinosad is a macrolide isolated from the fermentation of the actinomycete, *Saccharopolyspora spinosa*. Spinosad most notably displays commercial levels of insecticidal activity against the insect orders Lepidoptera, Thysanoptera, Diptera and select Coleoptera. It has been repeatedly embraced and recognized by a wide variety of governmental and publicly funded organizations as an insecticide of choice including those from the organic community. Here's a sampling of some key areas of recognition.

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Presidential Green Chemistry Challenge Awards Program

The Presidential Green Chemistry Challenge Awards Program is a governmental program which grants annual awards in recognition of innovations in cleaner, cheaper, smarter chemistry that incorporate principles of green chemistry into chemical design, manufacture, and use, and that have been or can be utilized by industry in achieving pollution prevention goals *(1)*. In 1999, the US Environmental Protection Agency (EPA) awarded the "Designing Safer Chemicals Award" to Dow AgroSciences for isolating, identifying, characterizing insecticidal activity, then developing the unique macrocyclic lactone, spinosad, from the microorganism *Saccaropolyspora spinosa. (2)*.

Interregional Research Project #4 (IR-4) Choice

Over the last several years, the publicly funded Interregional Research Project # 4 (IR-4) has been heavily involved in spinosad label expansion. IR-4's mission is to provide pest management solutions to growers of fruits, vegetables, other specialty crops grown on fewer than 300 thousand acres. IR-4 conducts residue trials required to expand labeled uses for these minor use, but economically important crops. Afterwards, IR-4 petitions the US EPA to establish tolerances or exemptions for a pest control product or crop. As part of their Biopesticides program, IR-4 has prioritized spinosad in food crop uses. In 2000 alone, 45 new tolerances that support 165 new spinosad uses were made available to growers through the IR-4 project *(3)*.

Organic Materials Review Institute (OMRI)

OMRI is a 501(c) (3) nonprofit organization created to interpret the newly established National Organic Program (NOP) standards established by the United States Department of Agriculture (USDA) for the organic community and the general public (4). OMRI's primary mission is to publish and disseminate generic and specific (brand name) lists of materials allowed or prohibited for use in the production, processing and handling of organic food and fiber.

OMRI (*5*) lists five different Dow AgroSciences products containing the active ingredient spinosad (85% spinosyn A [CAS Registry No 131929-60-7] and 15% spinosyn D [CAS Registry No 131929-60-0]) as "allowed" for use in organic agriculture: two versions of Conserve® insect control, Entrust® insecticide (which is a new organic formulation for crop uses), GF-120® NF Naturalyte® fruit fly bait and Justice® insecticide. In addition, there are other commercial offerings of an allowed product also containing spinosad by Green Light Co. and Woodstream Corp.

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Aerial Emergency Spray Choice

GF-120 NF fruit fly bait (containing spinosad) was used in an emergency aerial spray program for a quarantined area in San Diego County to fight a heavy infestation of Mexican fruit flies. Aerial spraying was approved in early January of 2003 for a 28-sq. mi. core of the 117-sq. mi. quarantine area in San Diego County to fight a heavy infestation of Mexican fruit flies *(6, 7)*. Approval was gained for spraying at two-week intervals for at least six months to be followed by releases of millions of sterile flies. Spinosad was selected by the State of California as the insecticide of choice for both organic and conventional agricultural areas.

Why Is Spinosad Attractive for the Organic Community, the US EPA and Third-Party Public Research Objectives?

Spinosad represents the combination of an efficacious product with biological natural origin coupled with a reduced risk safety profile. Efficacy information is not the focus of this chapter, but plenty of information on spinosad's efficacy and applications has been developed and thus is available for the organic community *(8-11)*. Likewise the details on spinosad's natural origin have been documented *(12, 13)*. This chapter will therefore focus on four topics: How is organic certification established in the US and how does this relate to safety considerations for spinosad and other organic products? What registration information is available for spinosad and other registered organics? What do labeling safety statements reveal about spinosad and other organic insecticides? What environmental and human safety assessment information supports the use of spinosad?

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Part I: How Is Organic Certification Established in the US and How Does this Relate to Safety Considerations for Spinosad and Other Organic Products?

The primary requirement for pesticides to be listed with "organic" status is that the pesticide is "natural" in origin. Consequently, safety by itself is not the key determining factor, although it is definitely a consideration. To understand the interplay of the primary and secondary objectives, a review of how the certification process is implemented is described below.

The current USDA National Organic Program (NOP) is established on principles found in "The Organic Foods Production Act of 1990" (*14*). The regulations provide consistency for use of the term "organic" as a means of protecting the consumer. As of October 2002, food labeled "organic," must conform to these organic production and handling standards. The guiding principle for organic production is that natural (non-synthetic) substances are allowed and synthetic substances are prohibited. A key component of the implementation requires the Secretary of Agriculture to establish a National List of Allowed and Prohibited Substances which identifies synthetic substances "that may be used, and the nonsynthetic substances that cannot be used, in organic production and handling operations." *(14)*. The lists allow organic producers to adhere to the stated primary goal of "organic" certification program regarding origin of the material and the principle that natural (non-synthetic) substances are allowed in organic production and synthetic substances are prohibited. There are also specific prohibitions regarding the use of genetic engineering, ionizing radiation, and sewage sludge in organic production and handling. The program also allows for specific exceptions to the general rules, primarily through the process of review.

While the NOP list categorizes materials by groupings such as allowed or prohibited materials, it does not translate generic materials (or active ingredients) down to formulated commercial products for the farmer or consumer. This is where the Organic Materials Review Institute (OMRI) as well as other certifiers has filled a role to publish and disseminate generic and specific (brand name) lists of materials allowed and prohibited for use in the production, processing, and handling of organic food and fiber. Their website *(4)* posts highly consulted lists (both domestically and internationally) for "organic" recommendations. Many manufacturers are advertising the fact they have received OMRI approval for their product. This is especially important since until recently EPA would not allow manufacturers to list organic approval on their Federal Pesticide Labels.

OMRI classifies products as Allowed, Regulated or Prohibited (A, R, P). Allowed materials may be used on certified organic land and crops; there are ~690 allowed products listed by OMRI (as of May 2004). Regulated materials may be used on certified organic land and crops only with certain restrictions;

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many can only be used after preferred, more natural alternatives were attempted as documented in an Organic System Plan. There are ~289 regulated products. Prohibited (P) materials may not be used on certified organic land or crops growing on that land for at least three years prior to the harvest of any organic crop from that given parcel.

Perusal of the Regulated List (substances that are allowed but with restrictions) is revealing with regard to the primary stated objective of natural versus synthetic as well as understanding how safety is considered. In general, the Regulated designation indicates there is a use restriction from the USDA NOP rule. A number of regulated materials can be used only after preferred more natural alternatives were attempted and their use documented in an Organic System Plan; basically these regulated substances represent rescue alternatives for the organic farmer and a type of tiered approach to "organicness".

Chemists schooled in traditional classifications of "organic" vs. "inorganic" will find intriguing that the regulated list includes many *inorganic* chemicals of natural origin (e. g. calcium chloride, potassium sulfate, sodium molybdate). The Regulated List also includes nonsynthetic herbicides, manures and fertilizers and even some synthetic micronutrients and regulated adjuvants. It is in this restriction of some items on the regulated list that signals an underlying and practical safety aspect. For example, some of the more toxic of the products which are technically natural in origin appear on the Regulated List; manure products are regulated due to potential microbial hazards.

As an exercise for understanding spinosad relative to other certified organic materials, Table I provides a summary of the OMRI findings for spinosad and several organic substances used as insecticides. Viewed through an organic lense, the spinosad products and *Bacillus thuringiensis* products are not restricted on the OMRI list, but are fully "Allowed" substances. Neem Oil extract and pyrethrum are "Regulated" materials. And interestingly, rotenone and sabidilla, which are often recommended on organic-focused websites *(15, 16)*, are not listed at all as of 2004.

These OMRI ratings are based primarily on origin, and not on a particular safety profile. In summary, the USDA National Organic Program website states: "USDA makes no claims that organically produced food is safer or more nutritious than conventionally produced food. Organic food differs from conventionally produced food in the way it is grown, handled, and processed." *(17)*. Understanding these principles allows a deeper understanding of what organic is and what it is not as implemented in the US.

Part II: What Registration Information Is Available for Spinosad and Other Registered Organics?

To understand the somewhat unique position of spinosad as organic requires an exploration of typical registration paths for pesticides in the US. As in Part I,

Table I. OMRI classification of spinosad and other insecticidal products

Active/ material	*OMRI classification*	*Example brand names*	*Natural Source*	*OMRI listing*
azadirachtin	neem extract	Neemix 4.5	derived from neem tree seeds	11 regulated
Bacillus thuringiensis	*Bacillus thuringiensis*	Dipel-DF	rod-shaped, endospore-forming aerobic bacteria	12 allowed
pyrethrum	botanical insecticide	PyGanic EC 5.0	derived from chrysanthemums	5 regulated
rotenone	botanical pesticide	Bonide rotenone 5% dust	extracts from plant roots of *Derris* spp., *Lonchocarpus* spp, and *Tephrosia* spp.	In theory approved, but no products listed
sabidilla	botanical pesticide	-	lily plant extract	none listed
spinosad	biological control	Entrust®	isolated from soil organism *Saccharopolyspora spinosa*	7 allowed

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a cross section of several known organic products relative to the EPA's registration process for pesticides was canvassed primarily through an internet search of general sources of registration information publicly available to the interested end-user. Spinosad was discovered to be atypical because it was first registered as a conventional insecticide with subsequent efforts focused on gaining appropriate "organic" status.

EPA registers pesticides under a statutory standard that requires determination of a "reasonable certainty of no harm" related to risks for human health and the environment. In order to make informed assessments, large sets of data are required from the registrant according to the EPA guidelines. There are two main paths for the registration of pesticides at the EPA: either conventional or biopesticides.

Conventional registration is more prevalent and involves submission of numerous registration studies conducted according to prescribed guidelines and protocols (typically 120 or more studies) which are used by the EPA's Registration Division (RD) to evaluate whether a pesticide has potential to cause adverse effects on humans, wildlife, fish, and plants, including endangered species and non-target organisms; evaluate environmental behavior including potential for contamination of surface or ground water from leaching, runoff, and spray drift; and evaluate human risks assessed for short-term and long-term (e.g., cancer, reproduction) effects based on estimated worker, bystander, and consumer exposures *(18)*.

Alternatively, registrants may pursue registrations through the Biopesticides and Pollution Prevention Division (BPPD) established in 1994 for products that fit the EPA's definition of a biopesticide: "certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals." Biopesticides fall under 3 main categories: 1) Microbial pesticides which consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient; 2) Plant-Incorporated-Protectants (PIPs) which are pesticidal substances that plants produce from genetic material that has been added to the plant; 3) Biochemical pesticides which are naturally occurring substances that control pests by non-toxic mechanisms *(19)*. These biopesticides are viewed as usually inherently less toxic than conventional pesticides. Based on that premise, many guideline studies may be waived (in part based on scientific literature), including crop residue trials and associated analytical methods, chronic mammalian toxicity testing, and formal environmental fate testing *(20)*.

A comparison of spinosad to several organic products relative to the EPA's registration process for pesticides is summarized in Table II. Information from standard sources of registration information for pesticides were pulled from web searches on the internet. Sources included product labels and material safety data sheets (MSDS) from either the Vance Crop Division green book (formally C&P Press) *(21)* or a vendor's website (*22*), EPA Fact sheets *(23)*, EPA tolerance listings *(24*) and EPA RED (Reregistration Elibigibility Decision)

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documents *(25)*. In the US, the label is a legal document and it is a violation of federal law to use a pesticide in a manner inconsistent with the label. The search was therefore focused on general sources of registration information publicly available to the interested end-user.

Table II reflects that fact that spinosad products were originally registered through the conventional registration process and thus there is a full set of registration data available. Neem oil and Bt are clearly considered biopesticides, pyrethrum is presumably a biopesticide, and the status of rotenone and sabidilla is unclear but these are presumably also considered biopesticides.

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EPA Signal Words (based on Mammalian Toxicity Classification)

For EPA registered products, the warning signal word is determined by the most severe toxicity category assigned to the five or more acute toxicity studies or by the presence of special inerts *(31)* according to the following scheme: Toxicity Category I = DANGER; Toxicity Category II = WARNING; Toxicity Categories III & IV= CAUTION. Product label warning statements classify two of the products, Neemix 4.5 (*26*) and PyGanic EC 5.0 (*27*), as WARNING, and three as CAUTION: Dipel DF (*28*) rotenone dust (*29*), and Entrust® insecticide (*30*). The product label for Entrust® insecticide displays CAUTION, the lowest classed signal word available.

Tolerances and Monitoring

As a result of the registration path for spinosad, Table II reveals 1) a significant difference between spinosad and the other organics in regard to the establishment of tolerances set for food commodities and 2) analytical methods available to the government for use in residue monitoring *(32)*. Spinosad has tolerance values established for 150+ food commodities. The others have been exempted from tolerance in the Code of Federal Regulations Part 180 *(33)*. The primary reason cited for the neem exemption was its use as a botanical fungicide/insecticide/miticide. Biopesticidal exemption is implied in the notice for *Bacillus thuringiensis*. No reason was stated for the exemption of pyrethrum, sabidilla or rotenone. This is not surprising. A more general comparison of the BPPD list of biopesticides and the EPA tolerance database *(24)* reveals that of the registered biopesticides in food use over 95% of these materials have received food tolerance exemption status. The wealth of food safety information

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Table II. Registration information available for the subset of organic products

Active/ material	*EPA Registration Number*	*Example brand names*	*Label Signal Word*	*EPA tolerance*	*Monitored by PDP?*	*Listed in FDA's Pesticide Analytical Methods*	*EPA Fact sheet location*
azadirachtin	70051-9	Neemix® 4.5	Warning	exempt CFR 180.1161	No	No	under biopesticides
Bacillus thuringiensis	73049-39	Dipel®DF	Caution	exempt CFR 180.1011;1108	No	No	several under biopesticides
pyrethrum	1021-1772	PyGanic® EC 5.0	Warning	*exempt CFR 180.1001; 70 commodities for related pyrethins*	*No*	*No, but related pyrethins are*	*none*
rotenone	4-17	Bonide rotenone 5% dust	Caution	exempt CFR 180.1001	No	No	under reregistration
sabidilla	*Not archived on web*	*Not archived*	--	exempt CFR 180.1001	*No*	*No*	*none*
spinosad	62719-282	Entrust®	Caution	150+ approved commodities	Added in 2000	Yes PAM II	under new pesticides

for spinosad relative to the other organic materials is a direct result of taking the conventional versus biopesticide registration path.

Tolerances are limitations on trace amounts of pesticide residues that may be legally present in foods. Tolerances are also used in preliminary dietary risk (safety) assessments by the EPA as upper limits for potential dietary exposure. Tolerances are established based on residue trials with the highest legal use rates and shortest window of application timings relative to harvest as established on a product label. A risk assessment process for the proposed tolerances is used to ensure that anticipated residues will not pose human safety concerns. To determine overall compliance rates for tolerances in domestic and imported food commodities, USDA sponsors a national effort, the Pesticide Data Program (PDP), to monitor for a large suite of pesticide residues on a variety of crops. In order to monitor for those pesticides analytical methods must be available for crop residues. Tolerances are referenced as enforcement legal limits primarily by the Food and Drug Administration (FDA).

As a note, years of monitoring data on pesticide residues indicates that generally average residues are well under tolerance limits. For example the 2001 PDP report indicates that 44 percent of all samples had no pesticide residues, 24 percent contained one residue and 32 percent contained more than one. Residues which exceeded the tolerance were detected in 0.1 percent of the more than 12,000 samples tested in 2001.

Tolerances and residues are an interesting point of discussion between the organic and conventional community. On one hand, the OMRI site *(4)* reports that available data demonstrate that organic produce contains fewer pesticide residues than non-organic produce *(34)*. But it is interesting to note the data assessed was for residues of conventional pesticides, not of organic pesticides. While there may be a lower detection of conventional pesticide residues in organic produce, it is unclear if these results are primarily due to the fact that the majority of organic products have an exempt tolerance status (and therefore are not typically monitored). On balance, it is noted that many botanical insecticides do tend to break down rapidly in the environment, are comparatively non-toxic, and are purported to be used by a relatively small fraction of growers. The bottom line, however, is that little compound specific data is available on the frequency of occurrence and magnitude of concentration of organic pesticide residues in foods. Of the active ingredients listed in Table II, only spinosad is part of the PDP monitoring program (added in 2000).

It is also interesting to note the organic community does use the tolerance values of conventional pesticides to set a practical threshold for pesticide residues even in organic crops. A commodity can be certified organic as long as it does not contain more than 5% of the EPA tolerance level of a conventional residue. But as stated earlier the overwhelming majority of conventional crops do not contain tolerance levels of pesticides. So by this rule, a certified organic crop could contain 10% blending of a crop at half the tolerance or even a higher

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percentage of a nonorganic crop if the residue was lower. Because ~40% of the tested commodities contain no conventional pesticide residues, the 5% tolerance rule is an intriguing standard for ensuring true organic produce.

In the end, the extensive list of established tolerances for spinosad represents significant additional work on the part of the registrant, IR-4, US governmental Agencies and some regulatory challenges for the expansion to new uses. Ultimately however, the fact that over 150 food tolerances are approved for spinosad provides the end-user and consumer assurance that a thorough safety assessment of the approved food use patterns has been conducted.

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Part III: What Do Labeling Safety Statements Reveal About Spinosad and Other Organic Insecticides?

The cross section of organic products was evaluated relative to some available key safety statements. Information sources here were the same as in Part II with a focus now on MSDS and product label statements. Table III summarizes key findings. Sabidilla was omitted due to that fact that no product label was readily found.

Preharvest interval (PHI) information is lacking for some and stated for others. For spinosad, the PHI will vary depending on the specific use; behind the approved uses there are multiple crop residue trials which have been conducted to set tolerance values in conjunction with a specific PHIs.

For Worker Reentry Intervals (REI), Bt and spinosad have the shortest time of 4 hours. The neem oil and pyrethrum have 12 hours. It is noted that in general 12 hours is a default REI and can only be lowered if additional data are submitted to EPA to allow for a risk (safety) assessment to refine and potentially lower the time. Therefore the longer REI for the neem oil and pyrethrum is most likely an acceptance of the 12 hour default, but that is not clear from the label.

Surprisingly, two of the MSDSs located (pyrethrum and rotenone) do not state acute oral toxicity information. Acute oral toxicity data is a basic piece of information needed for hazard evaluation. It is known that some deeper understanding does exist for both compounds, but that is not apparent to the MSDS reader. This difference indicates a variation in the depth of readily available product information for organic products.

With the exception of the Bts, all products here have some stated impact on fish and aquatics. The Bt RED states that data support the absence of toxicity to fish, although there is some evidence of moderate toxicity to *Daphnia*; however, the MSDS and label do not reflect this.

In conclusion, a survey of the various publicly available safety and registration information reveals both a variety in scope of posted information and a variety in depth of the existing information. Spinosad appeared to be

Table III. Safety Information Available for the Subset of Organic Products

Active material	*Pre harvest Interval (PHI)*	*Worker Reentry Interval (hours)*	*MSDS Acute Oral Information*	*Human Health Statements Additional information*	*Aquatic Toxicology statements (Label or MSDS)*
Azadirachtin	None stated	12	LD 50> 5 g/kg	EPA Fact Sheet states risk to human health not expected	May be hazardous to fish & aquatic invertebrates
Bacillus thuringiensis	Up to day of harvest	4	LD_{50} >4 g/kg on MSDS	NOAEL 4.7E11spore/kg from EPA RED;	No statement
Pyrethrum	none stated	12	No acute toxicology data is available	Presumably similar for pyrethrins	Toxic to fish
Rotenone	1 day	For home ornamental, vegetable, & small fruit gardens	Not on MSDS	EPA IRIS database: NOAEL, 0.38 mg/kg/day	Toxic to fish
Spinosad	depending on crop short as 1 up to 14 days	4	LD_{50} > 5 g/kg	EPA Fact Sheet: NOAEL, 2.7 mg/kg/day	Toxic to aquatic invertebrates, (fish and mollusks on MSDS)

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unique among the surveyed organic insecticides because it had been registered with a full data set under the conventional (albeit reduced-risk) review track at EPA.

Part IV: What Environmental and Human Safety Assessment Information Supports the Use of Spinosad?

As demonstrated above, the atypical registration path for spinosad provides an organic option with a solid foundation of technical information and rigorous registration assessment as a conventional product. This section is a review of some of this key information for spinosad. First three main areas of research with data available for spinosad are summarized: mammalian testing, ecotoxicology testing, and environmental fate testing. From these, two areas of assessment and their conclusions are reviewed: environmental impact and dietary risk.

Data from registration studies are used to conduct risk/safety assessments by governmental regulatory agencies prior to the approval of a product. Key data in the process is for potential exposure (environmental fate studies) and potential hazards (toxicity tests). It is critical to understand that exposure alone does not equal risk. Hazard alone does not equal risk. Instead, risk is a function of the two. Registration data are collected to understand how to establish use patterns so that the potential exposure will not exceed hazard thresholds and harm is avoided.

Toxicity studies are used to identify potential hazards and then set limits for exposure. A key premise of toxicology is "the dose makes the poison," and there are threshold doses below which no adverse effects are observable. Data from the environmental fate (including food residue and worker exposure studies) along with physical property information are used to determine the potential for exposure (for individual use patterns). If the potential exposure is expected to exceed the exposure limits established from the toxicity information, the product is not approved. Mitigation measures and label changes such as increasing the time between applications, or restricting use to certified applicators may be required to modify use patterns and ensure that appropriate risk management is achieved.

Mammalian Toxicity

For spinosad, a complete set of mammalian toxicity studies has been produced *(35-38)*. Acute results are included in Table IV. It is observed that spinosad displays low acute toxicity to mammals given it's high LD_{50} values. Spinosad is slowly and poorly absorbed through skin. Spinosad has been tested for long-term effects and has not been found to cause tumors in laboratory animals or have potential to cause neurotoxicity. As a result the EPA determined no special sensitivity factors were needed to account for children. A battery of genotoxicity studies have demonstrated no mutagenic potential.

Based on the NOEL (no observed effect level) established in a 2 year dog feeding study of 2.68 mg/kg/day, a chronic reference dose (RfD) of 0.02 mg/kg/day for spinosad was defined by the US EPA *(39)*. No acute reference dose was deemed applicable. An independent evaluation of spinosad by the World Health Organization has recently confirmed these interpretations.

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Table IV. Toxicity of Spinosad to Mammals

Test	*Assay Test Subjects*	*Results*
Oral LD_{50} (mg/kg)	Rats Males/Females	3738 / >5000
Oral LD_{50} (mg/kg)	Mice	>5000
Dermal LD_{50} (mg/kg)	Rabbits	>5000
Inhalation LC_{50} (mg/L)	Rats	>5.2 mg /L air/4 hours
Eye Irritation	Rabbits	Slight ocular irritation that clears within 48 hours
Dermal Sensitization	Guinea pig	No sensitization
Skin Irritation	Rabbits	Very slight irritation

Ecotoxicology

Spinosad registration data include testing on birds, fish, aquatic invertebrates, bees, earthworms, algae and non-target plants. Key results have been previously discussed by Cleveland *et al.* *(40)* and representative results resummarized in Tables V through VII. Spinosad is classed as slightly toxic to birds by EPA *(41)*. Spinosad is moderately toxic to fish, but it is 1000 to 10000 times less toxic than most synthetic insecticides. It is moderately toxic for aquatic invertebrates and classed as highly toxic to mollusks (however, see following risk summary). Although spinosad has been shown to be inherently toxic to bees in laboratory tests, the results of field and semi-field studies show the impact on bees is eliminated once the sprayed material has dried *(42)*. These studies have demonstrated that the hazard to bees is mitigated if spinosad is applied during periods of bee inactivity or if the hives are covered during application. This understanding has been translated to the label instructions for growers *(43)*:

> ***This product is toxic to bees exposed to treatment for 3 hours following treatment. Do not apply this pesticide to blooming, pollen-shedding or nectar-producing parts of plants if bees may forage on the plants duing this time period.***

Environmental Fate

Organic agriculture embraces methods that minimize impact on the ecological balance of natural systems. The environmental burden from

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Table V. Toxicity of Spinosad to Birds

Species	*Test*	*Result*
Mallard (*Anas platyrhyncos*)	Acute Oral (mg AI kg^{-1} body wt^{-1})	$LD_{50} \geq 2000$
Mallard (*Anas platyrhyncos*)	Acute Dietary (mg AI kg^{-1} in feed)	$LC_{50} > 5156$
Bobwhite quail (*Colinus virginianus*)	Reproduction (One generation) (mg AI kg^{-1} in feed)	NOEC = 550

LD_{50} = Dose which is lethal to 50 of the test population; LC_{50} = Concentration which is lethal to 50% of a test population; NOEC = no observed effect concentration.

Table VI. Toxicity of Spinosad to Fish

Organism	*Result*
Acute Tests (static 96-h)	
Rainbow trout (*Oncorhynchus mykiss*)	LC_{50} = 30.0 mg/L
Sheepshead Minnow (*Cyprinodon variegatus*)	LC_{50} = 7.9 mg/L
Sub-chronic Tests	
Rainbow trout (*Oncorhynchus mykiss*)	Early Life Stage NOEC = 0.498 mg/L
Rainbow trout (*Oncorhynchus mykiss*)	21-day flow through LC_{50} = 4.8 mg/L; NOEC = 1.2
Sheepshead Minnow (*Cyprinodon variegatus*)	Early Life Stage 35-d flow through; NOEC = 1.2

Table VII. Toxicity of Spinosad to Aquatic Organisms

Organism	*Test*	*Result*
Water Flea *(Daphnia magna)*	48-h; 24-h instars	LC_{50} >38.4 mg./L
Water Flea *(Daphnia magna)*	48-h; static renewal	LC_{50} >92.7 mg/L
Green algae (*Selenastrum capricornutum*)	7-d	EC_{50} >105.0 mg/L
Blue green algae (*Anabaena flosaquae*)	5-d	EC_{50} = 8.1 mg/L
Freshwater Diatom (*Navicula pelliculosa*)	5-d	EC_{50} = 0.14 mg/L
Grass shrimp (*Palaemonetes pugio*)	96-h	LC_{50} >9.8 mg/L
Midge (*Chronomus riparious*)	25-d static	EC_{50} >3.2; NOEC = 1.6
Eastern oyster (*Crassostrea virginica*)	96-h new shell	EC_{50} = 0.295 mg/L

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pesticides is reduced when compounds break down rapidly after application and thus do not have an opportunity to accumulate in unintended environmental compartments. One of the most important attributes of spinosad is its rapid degradation by multiple mechanisms (*40, 44-46*).

Spinosad is non-persistent with observed field dissipation half-lives ranging from 0.3 to 0.5 days (*35*). Primary pathways of degradation are photolysis by sunlight and microbial breakdown. Breakdown of spinosad exposed to sunlight has been observed in all key environmental compartments: treated plant surfaces (half-lives ranging from 2 to 16 days), water (half-life < 1day) and on bare field soil (<1 day). In the absence of sunlight, spinosad still undergoes microbial decay; laboratory studies conducted in aerobic soil in the dark indicate a bi-phasic degradation pattern with an initial half-life on the order of two weeks. A study under forestry conditions resulted in 50% dissipation times (DT 50 values) from 2.0 to 7.8 days (*45*): these results illustrate the timely breakdown of spinosad even with attenuated light.

Table VIII. Summary of Environmental Testing Results

Test	*Half-life*
Photolysis in water	<1 d
Field degradation	<1 d
Photolysis in soil	9-10 d
Photolysis on leaf surface	1.6-16 d
No sunlight; aerobic soil conditions (25 °C)	9-17 d
Water anaerobic conditions	161-250 d
Hydrolysis	Stable @ pH 5 & 7; 200-259 d @ pH 9

The environmental burden from pesticides is also reduced when compounds are not highly mobile. Compounds that stay put in the environment do not have an opportunity to move to groundwater or unintended habitats. Spinosad is moderately to strongly sorbed by soil particles and therefore is relatively immobile. K_d values from spinosad range from 4.3 to 32 mL/g depending on soil type and length of contact with soil. Both laboratory soil column studies and field studies have confirmed that spinosad has a low probability of leaching.

Ecological Risk Assessment

Detailed information on a tiered ecological risk assessment for the use of spinosad using ecotoxicology data, spinosad high use pattern and environmental properties has been presented previously by Cleveland *et al.* (*40*). The conceptual model assumed off-site transport of spinosad for unintentional exposure to non-target organisms. Tiered procedures communicated by the US EPA Environmental Fate and Effects Division (EFED) of the US EPA were used (*47*). In general, risk assessment involves comparison of potential exposure to potential hazard. Ecological risk is expressed as a risk quotient (RQ). The RQ is derived by calculating an estimated environmental concentration (EEC) that is then compared to appropriate toxicological endpoints such as the LC_{50} or the NOEC as follows.

$$RQ = \frac{\text{EEC}}{\text{Toxicological Endpoint}}$$

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The ecological risk assessments resulted in a conclusion of little concern for the majority of species tested, but a deeper understanding for effects on aquatic organisms and bees was needed.

Spinosad RQs did not exceed Tier I Levels of Concern (LOC) values for groundwater, mammals and birds or acute risk to aquatic organisms. Tier I methods did result in exceedence of LOCs for chronic exposures to aquatic organisms. A refined Tier II assessment of chronic risk from drift and runoff of spinosad into surface water was performed using regulatory modeling tools of GLEAMS *(48)* and EXAMS *(49)*. This refined assessment used fewer default values and took into account more detailed product-specific information available on the environmental behavior of spinosad. The results suggested only a small likelihood for spinosad water concentrations to exceed the chronic toxicity endpoint for *Daphnia*.

Current product registration label statements (based only on toxicity hazard classification system) state that spinosad is highly toxic to bees and mollusks. Tier I and refined Tier II modeling, standard and non-standard toxicity experiments, and field observations indicate that actual impact under field conditions for either aquatic organisms or bees can be effectively managed through grower spray drift minimization practices, notifying beekeepers, and avoiding spray applications during pollination.

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Dietary Assessment

Information related to dietary assessment is one aspect in which spinosad is truly unique from the other organics canvassed. A full data set on residues at harvest for multiple crops exists, and the spectrum of spinosad uses has rapidly expanded. Spinosad has been approved for use on over 150 crops, and it has a baseline 0.02 ppm tolerance for all commodities based on the GF-120 fruit fly bait (*50*). Elanco Animal Health has supported the registration a pour-on treatment or dilutable spray for cattle, and a dilutable spray for agricultural premises.

Originally, Tier I (screening level) assessments were conducted for spinosad using the very conservative assumption of 100% crop treated and use of tolerance values within the residue file. However, given the popularity of spinosad especially with many minor crop expansions from IR-4, a refined, more realistic assessment was needed. The standard Dietary Exposure Evaluation Model (DEEM) (*51*) software was used to assess and refine exposure for dietary levels of spinosad for the US population and subpopulations.

Both Tier II and Tier III levels of refinement for the dietary assessment for spinosad have been conducted at the request of the EPA. Tier II information included: 1) average residue values from magnitude of residues (MOR) crop trials to replace the use of tolerance values (known to be high-end limits); 2) bridging information for secondary commodities, using a surrogate average residue from within the crop group according to EPA crop rules; 3) spinosad processing factors; 4) animal dietary burden estimates based on worse-case animal diets. Tier III analysis included market share adjustments and refinement of anticipated dietary burden from animal commodities following dermal uses on cattle.

For both Tier II and III refinements, the chronic reference dose (RfD) of 0.02 mg/kg/day (defined by the US EPA) (*41*) was not exceeded for any US subpopulation. Table IX highlights information on the Tier II and Tier III exposure assessments. The highest modeled exposure was estimated to be for children in the age group of 1-6 years of age. This assessment was still very cautious because conservative assumptions were made when no data was available. These results indicate that with refined inputs there is adequate room in the FQPA dietary risk cup for existing and future new uses of spinosad.

Table IX. Percentage of Chronic Reference Dose Due to Dietary Exposure

Population Subgroup	*Tier II Exposure (Percent of RfD)*	*Tier III Exposure (Percent of RfD)*
U S Population (total)	11.1	4.3
All infants	13.6	6.2
Nursing infants	2.9	1.3
Non-nursing infants	16.8	7.7
Children 1-6 yrs	26.1	9.7
Children 7-12	15.7	5.9

Conclusion

A survey of the various publicly available safety and registration information sources revealed variations in the scope and depth of available information about certified organic pesticides. Of those certified organic products canvassed, spinosad had the most complete data set. Detailed, published risk assessments are available for spinosad, and relevant safety standards have been established (e.g., tolerances). The safety profile of spinosad compares favorably with that of other certified organic products.

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Chapter 9

Building a Multi-Tactic Biologically Intensive Pest Management System for Washington Orchards

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Tree fruit production has historically used more “high risk” insecticides than other agricultural systems and therefore has been significantly impacted by implementation of the Food Quality Protection Act of 1996. The key to transforming an agricultural system lies in developing alternative management approaches for key pests. The codling moth (CM), *Cydia pomonella* L., is a key pest in western apple and pear orchards. In the early 1990s, research demonstrated that pheromones could be used to manage CM. This knowledge led to the establishment of a USDA sponsored project known as the Codling Moth Areawide Management Program (CAMP). CAMP reduced crop losses and use of broad-spectrum pesticides while speeding the adoption of pheromones as a control tactic. Since that time, scientists that were associated with CAMP have been evaluating new technologies for pheromone delivery and other tactics, including soft insecticides, which strive to stabilize pest management systems in orchards. The goal is to maximize biological controls while minimizing impacts on human health and the environment.

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

The western United States produces most of the nation's fresh market deciduous tree fruits. For example, Washington State is the number one producer of fresh market apple, sweet cherry, and either number one or two for pear (*1*). The management of tree fruit pests in the western United States is simplified relative to fruit production in eastern regions because of habitat and climate. The relatively cold winters, especially in the Pacific Northwest, help synchronize pest development by eliminating all but the most hardy overwintering life stage. In addition, most western tree fruit crops are grown in areas with low summer precipitation (less than 30 cm per year). The lack of summer precipitation reduces problems from plant diseases that must be dealt with annually in eastern fruit producing states. The habitat surrounding most western orchards is primarily a semi-arid shrub-steppe. As a result suitable host plants for most insect pests are lacking, reducing the problems associated with their immigration into orchards. Because orchards are irrigated and incident solar radiation levels are high, trees can be managed intensively and production levels are high. The combination of climate, habitat, and intensive management offers a unique advantage to the western states for producing fruit organically or in a "biologically intensive" manner. Since most of our experience is with the Washington State fruit industry we will use examples from this production system, primarily from apple, to tell the story of how pest management programs have changed over time, what they are like at present, and where they are most likely heading.

Changes in Pest Management Programs

History of apple production in Washington State illustrates the evolution of a system dependent on synthetic organic insecticides to one that is now implementing a multi-tactic biologically based approach and supports the highest level of organic tree fruit production in the United States. Crisis often precipitates changes in management systems, and such was the case in Washington State in the 1960s. Reliance on chlorinated hydrocarbon insecticides (e.g., DDT) following World War II for control of the region's key pest, the codling moth, *Cydia pomonella* L., resulted in increased problems with spider mites, specifically the McDaniel spider mite, *Tetranychus mcdanieli* McGregor, and European red mite, *Panonychus ulmi* (Koch). Specific miticides were employed to control spider mites, but resistance to the miticides developed rapidly. It was common in mid- to late summer for foliage in apple orchards to take on a brownish cast due to injury by spider mites, despite the applications of several miticides. The crisis faced by the growers provided the environment allowing a paradigm shift in pest control tactics. Dr. Stan Hoyt (Washington State University, Tree Fruit Research and Extension Center) observed that spider mite problems were reduced or eliminated in certain orchards that used

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lowered rates of organophosphate (OP) insecticides. His research showed that the western predatory mite, *Galandromus occidentalis* (Nesbitt), could tolerate low rates of certain OP insecticides and provide biological control of spider mites and further, that these low rates of OP insecticides provided adequate control of the codling moth (*2*). The research in integrated mite management culminated in what is still recognized as a major breakthrough in pest management. Growers rapidly adopted the principles of integrated mite management, and by the end of the 1960s, most Washington growers had stopped applying specific miticides in apple orchards, relying instead on biological control of spider mites (*2*).

In the 1970s, the concepts of pest management were being elucidated and adopted in several cropping systems, including tree fruit (*3, 4*). Integrated mite management produced a stable apple pest management program with successful biological control of spider mites occurring in most Washington orchards. Codling moth was controlled with an average of about two applications per year using rates below the maximum allowed on OP insecticide labels (personal communication, S. C. Hoyt). Resistance to OP insecticides began to develop in some secondary insect pests such as the white apple leafhopper, *Typhlocyba pomaria* (McAtee), and apple aphid, *Aphis pomi* (De Geer); however, these pests were controlled with insecticides at relatively low rates and in a manner that did not disrupt biological control of spider mites.

In the 1980s, there was erosion in stability of the apple pest management program. Two leafroller species, *Pandemis pyrusana* Kearfott and *Choristoneura rosaceana* (Harris), appeared as serious problems in some orchards (*5*). The increased problem with leafroller pests was tied to a reduced efficacy of certain OP insecticides, especially chlorpyrifos (*6*). Also, a new pest appeared, the western tentiform leafminer (WTLM), *Phyllonorycter elmaella* Doganlar & Mutuura. The increase in pest status of the WTLM was most likely associated with the development of populations resistant to OP and most carbamate insecticides. The only effective insecticide against WTLM was found to be oxamyl, a carbamate insecticide that was also highly toxic to the western predatory mite. Thus, the WTLM problem added to the erosion of integrated mite management in some orchards. Stability returned to apple pest management programs when research showed that a small parasitic wasp, *Pnigalio flavipes* (Ashmead), was an effective biological control agent of WTLM and that it was tolerant of certain OP insecticides (*7, 8*). Codling moth control using OP insecticides was still effective; however, by the end of the 1980s the average number of insecticide applications used to control this pest had risen to almost three per year (Table I). There was interest in introducing synthetic pyrethroid insecticides into the apple pest management system during the 1980s, but recognition of their detrimental impact on integrated mite management (*13*), and pest management in general, resulted in growers rejecting use of these products for pest control.

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Table I. The average number of times an insecticide was applied per year and percent area treated ( ) in Washington apple orchards 1989-2001

Pesticide	*1989*[1]	*1991*[2]	*1993*[2]	*1995*[2]	*1997*[2]	*1999*[2]	*2001*[2]
azinphos-methyl	2.9 (98)	2.8 (90)	3.3 (81)	3.3 (94)	2.9 (91)	2.3 (78)	2.0 (73)
chlorpyrifos	1.3 (56)	1.4 (65)	1.3 (85)	1.3 (80)	1.4 (91)	1.3 (65)	1.1 (68)
ethyl parathion	1.2 (42)	1.0 (32)	0	0	0	0	0
methyl parathion	1.1 (17)	1.5 (28)	1.2 (24)	1.2 (19)	2.0 (33)	1.1 (5)	0
phosmet	2.4 (4)	2.1 (9)	1.1 (19)	2.4 (2)	1.2 (1)	2.0 (7)	1.5 (18)
petroleum oil	1.1 (90)	1.1 (88)	1.1 (88)	1.0 (77)	1.2 (87)	1.8 (69)	1.6 (79)
phosphamidon	1.8 (74)	1.2 (72)	1.4 (67)	1.4 (9)	1.4 (2)	0	0
imidacloprid					1.4 (65)	1.2 (50)	1.2 (38)
Bacillus thuringiensis	5.0 (<1)	0	1.9 (24)	2.2 (21)	1.5 (26)	2.0 (19)	1.2 (12)
spinosad						1.4 (39)	1.3 (50)

[1] Data from pesticide use survey conducted in Washington State (*9*).

[2] Insecticide usage data for Washington apple orchards from biennial surveys conducted by the USDA-NASS (*10, 11, 12*).

In the early 1990s, growers were facing increasing difficulties controlling codling moth, and resistance to certain OP insecticides, especially azinphosmethyl, was reported (*14, 15, 16*). The increased problem controlling codling moth was reflected in the gradual increase in the average number of azinphos-methyl applications per year (Table I). Problems with leafrollers occurred in more orchards (*17*). Research provided growers with control alternatives for these pests that would not disrupt biological control of spider mites, WTLM and other pests (*18*).

Concern about the impact of agricultural chemicals on infants and children (*19*), the environment, and residues on food fueled public debate and scientific inquiry. Regulatory action soon followed when the United States Congress passed the Food Quality Protection Act of 1996. This legislation required that all registered insecticides, and those proposed for new registration, be reviewed using criteria based only on the risks they posed to human health. Higher standards for risk assessments were used, including considerations of non-food uses of pesticides and additional safety factors for the assumed higher sensitivity of children and infants to pesticides in food. The Environmental Protection Agency established a priority to review those pesticides deemed most toxic to humans, the OP and carbamate insecticides. Because these products still formed the majority of insecticides used on tree fruit crops in the 1990s, increased interest was generated in finding alternatives for pest control.

Research on the use of mating disruption (pheromones) as a viable alternative for controlling pests in fruit crops was stimulated by success against the oriental fruit moth, *Grapholita molesta* (Busck) (*20. 21*) and promising results against the codling moth (*22, 23*). In 1995, the Codling Moth Areawide Management Project (CAMP) was initiated in three states. This was a cooperative effort between the USDA-ARS and three land grant institutions: Washington State University, Oregon State University, and the University of California at Berkeley. CAMP established five demonstration sites in three states. CAMP documented substantial reductions in the use of OP insecticides directed at codling moth control while at the same time reducing crop losses (*24, 25*).

Howard Flat, located near Lake Chelan in Washington, is a good example of how the use of mating disruption at a CAMP site improved management of codling moth. Codling moth losses at Howard Flat were estimated to be about 0.9% in 1994, one year prior to the beginning of CAMP, with an average of nearly 30 codling moths per pheromone trap and 2.7 insecticide applications per year used for its control (Figure 1). As the areawide use of mating disruption plus supplemental insecticides took effect, the average number of codling moths per trap declined dramatically, as did the average percent crop loss. By the end of the third year (1997), the average crop loss due to codling moth was only 0.01% (Figure 1). The low level of crop loss was maintained during the following two years even while the average number of insecticides applied per year dropped to less than 0.5 (Figure 1). By the end of CAMP, the pheromone use by Washington apple growers had increased from 6,500 to almost 24,300 hectares treated. Implementing a pheromone-based pest management approach in CAMP initially resulted in increased problems with leafrollers, which were managed with less hazardous, non-OP insectcicides ("soft" pesticides), but not with other secondary pests (*26*).

The primary means of delivering pheromones for mating disruption of codling moth control has been via hand-applied dispensers. These dispensers are applied at densities from 500-1,000 per hectare. Pheromone evaporates from the surface of dispensers, and most last the entire season. Over a three-year period (2001-2003) we evaluated different hand-applied dispensers to characterize how they released pheromone. Dr. Vincent Hebert reviews this work in a chapter in this book (*27*).

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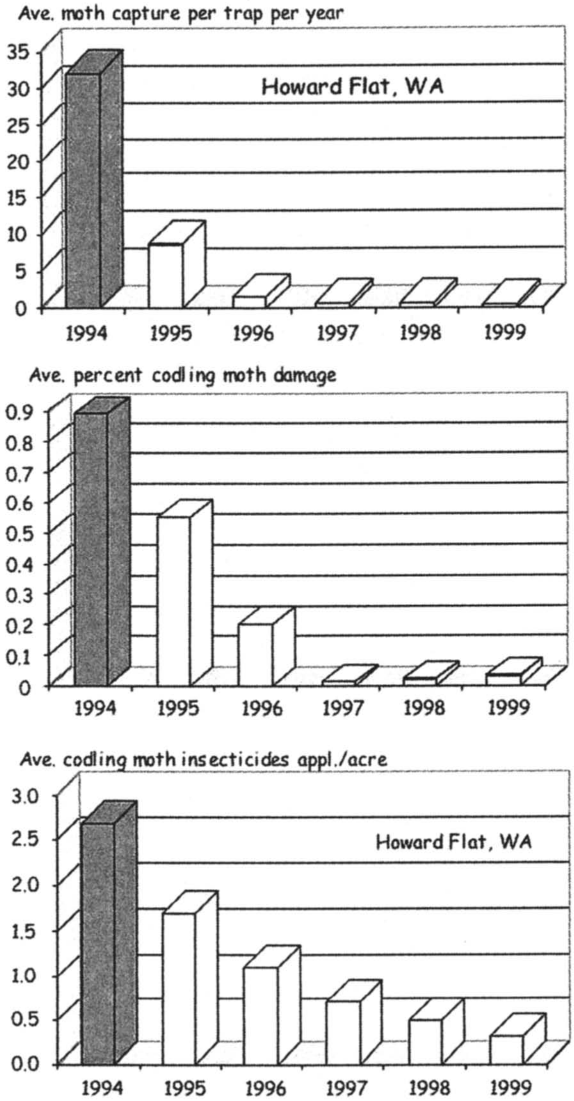
Ave. moth capture per trap per year

Ave. percent codling moth damage

Ave. codling moth insecticides appl./acre

Figure 1. Results from the Howard Flat CAMP site showing data on levels of codling moth adult activity, fruit injury and insecticide applications to control this pest prior to (1994) and throughout the project duration (1995-1999).

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In 2000, many members of the research and extension team who worked together in CAMP joined in two federally funded projects (*28, 29*). The goal of these projects was to refine and extend the benefits of a pheromone-based IPM system to additional apple and pear acreage and to extend this technology into walnut production in the western states. Scientists associated with these projects, (dubbed "Areawide II") are conducting research on new ways to deliver pheromones that would make them either less expensive to use or more effective. Efforts include using high-emission release devices, referred to as "puffers" or "misters." These devices release massive amounts of pheromone from a very few sites per area. Puffers have shown promise in apple orchards and walnut groves where in the latter, tree height is a challenge for more traditional pheromone delivery systems, i.e., the hand-applied dispensers (*30, 31*). Researchers are also evaluating methods of pheromone delivery such as sprayable (*32*) and hollow fiber formulations (*32, 33*). These formulations are the opposite of the "puffer" approach in that they release pheromone from thousands of sources per area, and they have the possible added advantage of being applied by air.

Research has clearly demonstrated that the use of mating disruption can reduce reliance on insecticides to control codling moth; however, they have not eliminated the need for insecticides as part of a pest management program. Growers are currently combining the tactics of mating disruption and insecticides to achieve acceptable levels of crop protection in apple and pear. This approach remains a barrier to a more robust biologically intensive pest management program because even the use of one OP insecticide can disrupt biological control of certain pests. The "Areawide II" team has demonstrated that alternatives to OP insecticides can be used for control of codling moth and other apple and pear pests without reducing high standards of crop protection. A recently completed three-year implementation project in 15 Washington apple orchards demonstrated that pheromones supplemented with only "soft" insecticides (those that do not negatively impact biological control agents) provided crop protection as good as pheromones supplemented with broad-spectrum insecticides. This efficacy was achieved at no increased cost to the grower (*34*). Results of this project suggest that many Washington apple and pear orchards could move away from use of OP insecticides, thus enhancing the opportunity for biological control of pests in their orchards.

Organic Fruit Production in Washington

The pest management continuum continues to an "organic" production end point. Organic production, while being holistic in including more than just insect pest management, is also highly legalistic. Only certain kinds of products and practices can be used in organic production, and growers must become certified to market their fruit with an organic label. The western US produces more organic apple, pear, and sweet cherry than any other region of the country (*35*). While as a percentage of the total apple acres in Washington State, production of organic and transition organic fruit remains small (≈5%), its growth over the last decade has been dramatic. Granatstein and Kirby (*35*) reported that in Washington State organic apple production (certified acres) increased from 1,200 in 1991 to 6,540 in 2001; plus, there were an additional 3,411 transition organic acres that year. Organic pear and sweet cherry production has also increased dramatically over this same period. There is a potential for a much greater increase in organic apple, pear and cherry production in western states with the registration of two new insecticides that will control codling moth and a key pest of cherry, *Rhagoletis indifferens* Curran. The greatest barrier to increased organic fruit production is the lack of a consumer demand that will support higher retail prices to offset the higher production costs of organic fruit.

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Conclusions

The historical perspective presented in this article shows that western apple orchards are moving along a pest management continuum from what can be referred to as a "conventional" approach that relies almost exclusively on synthetic organic insecticides towards a more "biologically intensive" system (Figure 2). Calls for more biologically intensive pest management programs arose from a symposium on Food, Crop Pests and the Environment sponsored by the USDA and EPA and held in Washington, D.C. in June of 2002 (*36*). The "biologically intensive" phrase added to pest management was an attempt to place more emphasis on developing multi-tactic approaches to crop protection that would allow a greater role of biological control in agricultural systems. Apple pest management programs in Washington have steadily moved from a traditionally conventional approach towards a more biologically intensive approach. Integrated mite management showed that there was a different way to think about apple pest management, but progress was slow. By the 1980s, more examples integrating biological and chemical control had been developed, and growers and crop consultants were using population monitoring and thresholds to make pest control decisions (*37*). Shifts in the apple pest management program are documented in pesticide use survey results over the last 12 years (Table I). Uses of some broad-spectrum insecticides, ethyl parathion and methyl parathion, have been eliminated because of regulatory action. An OP insecticide, phosphamidon, used primarily to control aphid pests, was replaced with a more

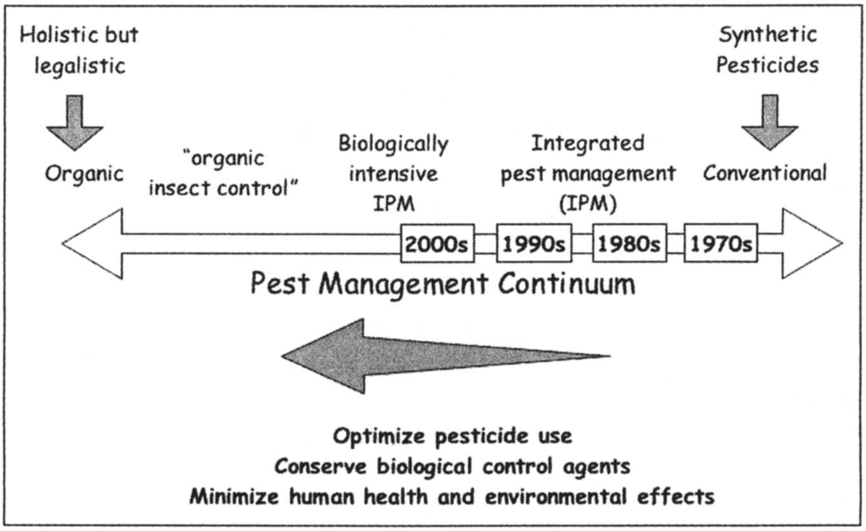
Holistic but legalistic
Synthetic Pesticides
Organic
"organic insect control"
Biologically intensive IPM
Integrated pest management (IPM)
Conventional
2000s
1990s
1980s
1970s
Pest Management Continuum
Optimize pesticide use
Conserve biological control agents
Minimize human health and environmental effects

Figure 2. A conceptual pest management continuum from programs relying only on synthetic insecticides as a control tactic (conventional) to ones that are holistic but highly legalistic (organic).

selective insecticide, imidacloprid, in the late 1990s. The use of *Bacillus thuringiensis* (*Bt*) increased in the mid-1990s as a "soft" insecticide solution to increased leafroller problems. In the late 1990s, spinosad, a new selective insecticide, was introduced for management of leafrollers (*38*). In the 1990s, the use of mating disruption was introduced, and adoption of this technology reached nearly 50% of apple acreage by the end of the decade. The use of mating disruption has remained fairly constant in Washington, even through very difficult economic conditions of the late 1990s and early years of the new millennium (Figure 3). The reduction in azinphosmethyl use for codling moth control between 1995 and 2001 (Table I) coincided with an increased adoption of mating disruption (Figure 3).

In the current decade, new insecticides are being introduced that will help replace or further reduce broad-spectrum insecticide use (*34*), and new ways of delivering pheromones promise to reduce the costs of this technology. A new areawide organic insect pest management program in pear has demonstrated not only protection of sensitive freshwater habitats from potential broad-spectrum insecticides, but also the added value of products grown in environmentally sensitive ways (*39*). In addition, scientists are examining the design of orchards and manipulating surrounding habitats to create refugia for natural enemies. For example, Dr. Thomas Unruh is working with growers to establish gardens of

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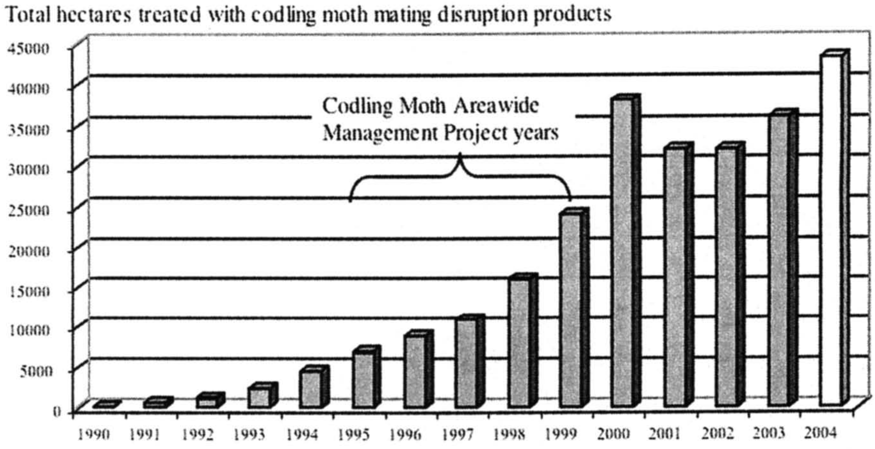

Figure 3. Estimates of the hectares treated with codling moth mating disruption products as part of a pheromone-based management effort in Washington apple and pear orchards from 1990 through 2004.

wild rose and strawberry that harbor a leafroller species, *Ancylis comptana* (Foelich), that provides an overwintering host for a key parasitoid, *Colpoclypeus florus* Walker, which is an important natural enemy of pestiferous leafroller species that inhabit orchards (*40*). Dr. David Horton has identified key plants in native habitats that harbor natural enemies that are important in suppressing pests in pear orchards (*41*). We are also developing new information on the seasonal occurrence of parasite species attacking leafroller pests, providing a means of more accurately determining their impact and identifying times of the year to avoid use of insecticides that would disrupt their activities (*42*).

Understanding how various biological components fit together into an interactive matrix can be daunting. To help us understand these interactions, Dr. Vincent Jones has developed a novel marking methodology that is being employed to assess movements of insect pests and their natural enemies between various components of the orchard ecosystem (*43*). Progress in developing and implementing biologically intensive pest management programs for apple and pear, and even walnut production, in the western United States is being made through the research and education efforts of many people (*28, 29*). As new technologies are developed, they are being evaluated and integrated into pest management programs that have high standards for crop protection. As we understand how complex biological systems interact on a spatial scale that is larger than an individual orchard, new approaches for managing pests as well as their natural enemies will be possible.

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Chapter 10

Evaluating the Pheromone Release Rate Characteristics of Commercial Mating Disruption Devices

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Mating disruption has become an important integrated pest management tool for controlling codling moth injury in apple and pear orchards in the Pacific Northwest. This area-wide management practice has recently aided in reducing reliance on traditional organophosphorus and carbamate pesticide control practices. Ideally, commercially produced hand-applied dispensers release pheromone throughout the growing season at constant release rates approaching zero-order kinetics. Unfortunately, the success of codling moth mating disruption has recently declined, especially during the pests second generation of the year. In part, this can be attributed to non-uniform chemical release behavior from field-aged dispensers under actual orchard conditions. This paper presents 2001 through 2002 chemical release characteristics of commercially available dispensers as they aged in the orchard over the ca. 4-month moth-mating season in North-Central Washington and North-Central Oregon. The goal of this 2-year program was to provide an unbiased and rigorous assessment of pheromone dispenser behavior under actual

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orchard conditions. A second goal was to provide realistic dispenser release information to the tree fruit industry for making prudent decisions for their pest management needs.

The Codling moth (*Cydia pomonella (L.)*, CM) is the key insect pest in Pacific Northwest pome fruit production (*1*). Traditionally, organophosphorus (OP) insecticides have been applied to orchards in the spring-summer period for controlling CM. Passage of the Food Quality Protection Act of 1996, however, will eventually restrict or possibly eliminate use of OP insecticides used for CM control. Besides restricting the use of Pennicap-M and post-bloom Lorsban, increased regulatory constraints on OPs such as azinphos-methyl and phosmet, incidences of resistance to these insecticides, and continuing problems with secondary pests have added urgency to the development and adoption of alternative pest management options.

In the early 1990s research at the USDA-ARS and Washington State University's Tree Fruit Research Extension Center demonstrated the potential of using sex pheromones for mating disruption as a tactic for controlling CM populations and reducing pome fruit injury in the Pacific Northwest. The USDA funded codling moth area-wide management program (CAMP) implemented from 1995-1999 showed that CM densities and fruit injury could be dramatically reduced when pheromones were used on an area wide basis. The adoption of this area wide program also resulted in greater than 70% reduction of OP insecticide use (*2*).

Constant release of the CM sex pheromone codelemone (Figure 1) from solid matrix dispensers can saturate the canopy air and effectively disrupt moth mating (*2*). Through the efforts of the American Semiochemical Association (ASA) codlemone was registered by the EPA as a reduced risk straight-chained lepidopteron pheromone (SCLP) and granted exemptions from food and feed tolerances.

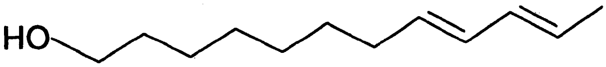

Figure 1. Structure of codlemone, (E, E) 8,10 dodecadien-1-ol

Codlemone's tolerance exemption status has greatly reduced the registration timeline for commercial dispenser products. What had taken years from the period of initial dispenser development through commercial viability could now occur in months (*3*). As for all agrochemical label uses, pheromone efficacy data must be generated supporting the claim that the registered product provides

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effective control. However, unlike insecticide active ingredients that are designed to control by ingestion or on contact, mating disruption delivery systems must be designed to provide an adequate and constant release of pheromone for season-long CM mating supression.

Many environmental factors such as altitude, air temperature, wind, and relative humidity can influence the rate of season-long release of pheromone from the dispenser. The vapor pressure of organic compounds similar in structure to codlemone should be expected to increase by a factor of 2 with each 10°C rise in air temperature (*4*). If not accounted for, higher seasonal temperatures can result in a more rapid chemical release from the dispenser. Therefore, a balance must exist between making a dispenser that will release enough pheromone to provide effective mating disruption and having one that will not be depleted over the entire moth-mating season (*5*). The commercialized design must also factor in changes in dispenser release performance due to field-aged weathering/oxidation of the dispenser material.

Because of difficulties and costs in acquiring chemical release data over the entire moth-mating season, information has in the past been generated from laboratory-aged dispensers that may not reflect chemical release under season-long orchard conditions (*6,7,8*). Often, formulation and dispenser material changes continue to be made to optimize seasonal dispenser release, especially when orchard growers report failures in moth mating disruption. This season-to-season trial and error approach in the end may lead to greater grower reliance on chemical control.

Of the various types of pheromone delivery systems that are commercially available, solid matrix dispenser systems are predominantly used in the Pacific Northwest. These hand-applied dispensers are all passive release devices that are placed in the trees at a rate of 100-400 dispensers per acre. Ideally, pheromone is released from these multiple point source emitters at a moderate rate for season-long mating suppression.

Currently, there are four major CM hand-applied dispenser manufacturers. Each manufacturer's dispenser design is markedly different—pheromone may be embedded in flattened membrane polymers, polyethylene tubes, or in plastic spirals. Each manufacturer claims their dispenser design meets the dose and controlled release requirements for season-long CM mating suppression.

Efficacy testing is performed to verify consistent season-long pheromone release and provide environmentally relevant dispenser release information to the fruit industry for making prudent decisions for their management needs (*5,6,8*). Solid matrix dispenser release performance has been evaluated by the following methods.

- Gravimetric methods (i.e., determining weighing loss from the field-aged dispenser at timed intervals (*6,8*);
- Total extractions of the field–aged dispenser to determine the residual concentration of the pheromone(s) remaining (*6,8*);

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- Non-destructive dynamic pheromone vapor trapping from the field-aged dispenser (*5,8*).

Estimating the residual pheromone concentration in the field-aged dispenser by gravimetric methods is inexact and problematic since the loss may not be from volatilization alone and chemical and photochemical oxidation cannot be accounted for by gravimetric methods (*6*). Moreover, field-aged dispensers have been observed to gain weight from water absorption or collection of debris on the dispenser surface (*5*). A more precise technique for estimating product release rates is total solvent extractions of field-aged dispensers. Essentially all the pheromone in a dispenser must be removed by an organic solvent or through total solvent digestion of the dispenser material. The amount of pheromone and possible oxidation and degradation products can then be determined for each field-aged dispenser using gas chromatography. This method assumes that the difference in the amount remaining at each time will be a measure of the amount of pheromone released. The day zero or initial amount, therefore, should reflect the amount of codlemone in the dispenser as stated by the registrant on the label. Whereas, the residual amount in the field-aged dispenser at time *t*, minus the pheromone concentration at time zero should reflect the amount released over that time interval.

Dynamic trapping of the chemical vapor from the field-aged dispenser provides the best comparative measurement of the release rates among different dispensers (*8,9,10*). In order to gather comparative release data among dispenser types, an important consideration is that every dispenser type must be subjected to the same laminar airflow characteristics at similar atmospheric pressure and temperature conditions. Unfortunately, dispenser release systems are not uniform in their sizes and shapes and can thus disrupt laminar airflow making dispenser release comparisons difficult to achieve.

Quantification of pheromones by either residual analysis or volatile trapping has been in the past performed by gas chromatography with flame ionization detection (FID). This chromatographic approach, although suitable for high pheromone concentration residual dispenser evaluations, is mass insensitive and thus requires extended volatile trapping loading times (i.e., sometimes > 1 day) and effluent from many replicate dispensers to acquire enough pheromone for analysis. Previous VT evaluations required that up to 5 field-aged dispensers had to be sampled in an air stream for 48-hours to acquire enough pheromone for GC/FID determination (*5*). Moreover, since the FID is a non-specific detector, it also has limited capability for evaluating possible compound decomposition products.

This paper summarizes residual pheromone evaluations from field-aged dispensers taken from apple orchards located in the Pacific Northwest in years 2001and 2002. The total pheromone from each field-aged dispenser was determined using GC/FID. Also summarized is year 2002 field-aged dispenser

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volatile trapping (VT) determinations on individual dispensers by gas chromatography with mass selective detection (GC/MSD). We found that using the novel technique of trapping volatilized pheromone with porous polyurethane foam (PUF) followed by GC/MSD determinations allowed individual field-aged dispenser release rate data to be reliably gathered in a much shorter volatile trapping collection period than has been previously reported. We also found that using these two dissimilar analytical approaches provide a reproducible and comparable assessment of pheromone dispenser behavior under actual orchard conditions.

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Methods

Aging of Dispensers

In 2001, five different solid matrix dispensers were field-aged by placing them in the orchard according to label instructions at a height of ca. 3 m within an apple orchard canopy located at the WSU-Tree Fruit Research & Extension Center (TFREC), Wenatchee, WA. In 2002, dispensers were field-aged in a similar fashion at two separate orchard locations, the TFREC orchard and a commercial apple orchard in Medford, OR. Enough dispensers were field exposed to allow collection of 10 dispensers of each kind approximately every 30 days in 2001 and at 14-day intervals in 2002. Five of each dispenser type destined for analysis in the VT (volatile trapping) system (Figure 2) or by residual dispenser extraction were placed together into a Mylar bag, sealed, labeled, frozen, and then sent to the WSU-Food and Environmental Laboratory (FEQL) for residual pheromone and VT system evaluations.

Residual Pheromone Extraction and Analysis

All of the pheromone remaining in a dispenser was solvent extracted and then quantified using GC/FID. Because each of the dispenser types were distinct in its polymer material and shape, a uniform extraction procedure was impossible to develop. Extraction procedures for different pheromone dispensers were provided from the manufacturers. Methods for the solvent extraction of Isomate C+ and Isomate CTT was provided by Pacific Biocontrol Corporation ("Analytical Method of Active Ingredients and Stabilizers in Codling Moth Pheromone Dispenser, 1999), method for Checkmate was provided by Consep Inc. ("Analytical Method for Checkmate CM Manufacturing Use Product and End-Use Product Via Gas Chromatography"), method for

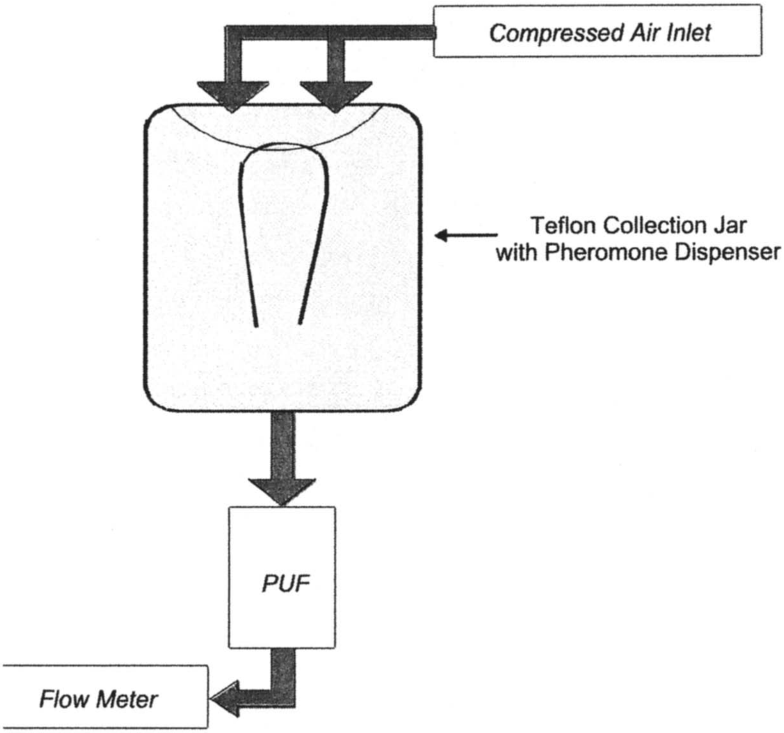

Figure 2. Volatile trapping system for measuring pheromone release from field-aged solid matrix dispensers

Disrupt CM Xtra was provided by Hercon Environmental ("Analytical Method for Hercon Disrupt CM Xtra") and method for Nomate was provided by Sentry Biologicals ("Gas Chromatography Method for Codling Moth Spiral Analysis").

Minor modifications to all of the above manufacturer methods, however, were introduced for the specific requirements of cross-comparing pheromone concentrations from all dispenser types in a consistent manner. In some instances, the dispenser type was immersed in a known amount of organic solvent then ultrasonicated and brought to a suitable volume for residue determination. In other instances, the dispenser material type was completely dissolved in organic solvent and adjusted to a suitable volume before residue determination.

For each of the five commercial dispenser types five replicate dispensers per field-aged interval date were extracted for subsequent analysis. Myristic

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acid methyl ester was added during the initial extraction to each sample as a recovery-surrogate standard. To directly compare residue concentrations, all replicate extracts was then brought up to a similar solvent volume

Some of the commercial dispensers contained a mixture of SCLPs together with codlemone, while others only contained just codlemone. Only codlemone release rates were compared among the five dispenser types. Codlemone was determined by gas chromatography using a 15 m x 0.53mm I.D. x 1.00 µm film thickness Carbowax phase glass capillary column using FID. Residual extractions were greater than 95% efficient based on recovery of the internal surrogate. The working method limit of quantitation for codlemone was estimated to be ca. 0.5-ppm.

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VT System

VT is a dynamic system that provides release rate data of a dispenser of a certain age under a set of airflow and temperature conditions. The VT system that was developed in our laboratory (Figure 2) involves passing purified clean air over individual dispensers and then trapping the pheromone released under constant conditions onto highly porous polyurethane foam (PUF) adsorbent over a 2-h period.

Compressed gas tanks provide a consistent source of breathing quality air. The compressed air was connected by tubing leading to a Y connector that splits the air into two inlets on the top of the Teflon collection jar. The tubing from the base of the collection jar was connected to a glass holder that contained the PUF adsorbent. An in-line flow meter (Gilmont) continuously monitored airflow through the VT system. In order to minimize chemical sorption to surfaces, all the tubing, connections, ferrules etc. leading from the collection jar to the glass PUF holder were constructed from Teflon. Five individual collection jars were prepared and connected onto a 5-port regulated air pressure manifold.

Prior to volatile trapping, field-aged dispensers were removed from the cold storage/freezer and allowed to equilibrate at room temperature for 22- 24 h. In four of the collection jars, an individual pheromone dispenser was suspended using Teflon tape. For each field-aged interval date, four replicate dispenser samples were positioned for volatile trapping. To determine material balance and assist in monitoring system performance on an individual VT system basis, a known amount of myristic acid methyl ester sorbed on glass filter paper was used as a surrogate. After volatilization, the surrogate was extracted from the filter paper, collection jar walls, and from the gas-phase PUF adsorbent. The use of the surrogate also assisted in evaluating the extraction efficiencies and to some extent possible leaks or other malfunctions of the system during the volatile trapping sampling time.

After connecting all system components, the dispenser and the surrogate-fortified filter paper were placed into the collection jar and the airflow ran at ambient room temperature (20°C ± 2°C) for 2 h, at a flow rate of 10 L/min. Flow rates and humidity of the air leaving the collection jar during sampling were measured and recorded. Air temperature within the Teflon collection jar was monitored and recorded automatically every 5 min with a Hobo® temperature device during the 2-h sampling period.

After trapping released pheromone, the PUF cartridge was removed from the glass holder and placed into a 100-ml wide mouth glass extraction jar. Extraction solvent (10% ethyl acetate, 90% hexane) was added to submerge the PUF and the sample was ultrasonicated for approximately 10 minutes. The extraction solvent was removed from the PUFs by filtration under vacuum and the solvent extraction/sonication steps repeated. The two solvent fractions were combined, concentrated, and then quantitatively transferred to 15-ml centrifuge tubes. The final volume was adjusted with hexane to an appropriate volume for GC determination. Solvent extracts were analyzed by GC-MS in total ion chromatography (TIC) mode.

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Results and Discussion

Residual Pheromone Analysis

Table I shows the results for residual codlemone evaluations performed on five dispenser types in 2001. We assume that the difference in the amount of pheromone remaining in the dispenser from one time interval in relation to the concentration at day-zero should correspond to the amount of pheromone released. The day-zero amount should also reflect the amount of codlemone loaded into the dispenser as stated by the registrant on the label. All the replicate day-zero residue information was in close agreement with labeled specified concentrations with the exception of one dispenser that was loaded with ca. 15% less active ingredient than specified.

Most field-aged dispensers showed a gradual loss of codlemone over time with the Isomate-C plus and Isomate CTT dispensers exhibiting a more consistent near zero-order gradual release. The Checkmate dispenser showed a residual codlemone pattern similar to the higher loaded Isomate CTT dispenser through day 56 but thereafter showed a higher residual content, and on day 162, it retained 38% of its original codlemone load. The Disrupt dispenser showed the most variable results in the residual analysis of pheromone. The information gathered on days 84 through 162 suggest that weathering of this dispenser material attenuated chemical release. The Disrupt dispenser also retained more

Table I. Residual codlemone concentration from field-aged dispensers

	Average amount (mg) of codlemone remaining per dispenser				
Age of dispenser	*Isomate C plus*	*Isomate CTT*	*NoMate CM*	*Checkmate CM*	*Disrupt CM*
0	140.43	292.78	110.58	289.17	176.30
35	111.50	243.68	54.09	243.14	148.75
56	96.66	220.52	38.16	226.90	139.24
84	82.29	171.47	15.07	194.56	106.64
112	46.62	128.31	5.35	184.90	109.72
148	35.47	97.32	2.35	123.99	87.47
162	24.45	88.34	1.7	108.81	81.10
% released	83	70	98	62	54

of its codlemone (i.e., 46% of its day- zero concentration) over the experimental timeframe, more so than any other dispenser type.

The original codlemone load of the NoMate dispenser, 110 mg per dispenser, was less than the labeled amount, 120 mg per dispenser (Table I). By day 35, less than 50% of the original codlemone remained in the dispenser and by day 84 only 14% of the original codlemone load was detected. This dispenser released its pheromone load early in the mating season, consistent with dose dependent first-order release behavior. Only 2% of the original pheromone amount was left in the dispenser by day 162.

The total amount of codlemone released in the orchard canopy on a per acre basis over the first (from days 0 through 84), and second codling moth mating flights (from days 84 to 161) were estimated (Table II). These estimates were based on the 2001 residual dispenser data but also assumed that maximum label rates were used in the orchard.

Table II. 2001 Residual Codlemone Concentration from Field-Aged Dispensers

	Estimated grams of codlemone released per CM flight				
CM generation	*Isomate-C plus*	*Isomate CTT*	*NoMate CM*	*Checkmate CM*	*Disrupt CM*
dispensers per acre	400	200	400	200	200
1st gen. flight	23.25	24.26	38.20	18.92	13.93
2nd gen. flight	23.14	16.63	5.35	17.15	5.11

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The amount of codlemone released per acre over the first mating flight for all dispensers was relatively constant and ranged from 14-38 g per dispenser type. Whereas, estimated release per acre over the period of the second flight dropped off sharply for certain dispensers. The amount released ranged from 5 to 23 grams. Assuming that some threshold concentration must exist in the orchard for efficacy, those dispensers that were either depleted early on or were not releasing chemical consistently after 80 days of field aging may not have been effective in suppressing CM mating during the second generation.

Figure 3 compares the residual concentrations of codlemone extracted from field-aged dispensers taken at the WSU-TFREC orchard during the two moth-mating generations in 2001and 2002. The Disrupt dispenser was not evaluated in 2002. The release behavior of the remaining four dispenser types was observed to be near similar to the 2001 residual concentration data, even when factoring in differences in seasonal conditions. Again, all field-aged dispensers showed a gradual loss of codlemone over the two moth-mating generations. The residual codlemone 2002 Checkmate membrane dispenser data, however, indicates a slower rate of release over the second moth-mating period. Conversely, the 2002 NoMate dispenser demonstrated improved product performance showing higher initial loading of codlemone in the dispenser, and higher residual amount towards the end of the 2-generation moth-mating season.

VT System

Starting in 2002, we developed the VT system to individually assess and compare release of pheromone from field-aged dispensers over a short interval under controlled airflow conditions in the laboratory. To verify the reliability of this system, we evaluated two dispenser types that were field-aged at the WSU-TFREC orchard. These two dispensers were shown by residual analysis to have comparable day-zero codlemone loadings but dissimilar field-aged chemical release behaviors. An important assumption of the VT method is that every dispenser type must be subjected to the same airflow characteristics. The positioning of dispensers in the 1-liter VT chamber could spatially influence laminar airflow. The Isomate and NoMate dispensers were well suited for VT comparison because their size and shape suggested that they were more likely to be uniformly exposed to airflow within the VT chamber system. Figure 4 shows the volatile trapping results conducted for these dispensers over a 2-h airflow-sampling period.

The Isomate C+ dispenser released its pheromone contents in a steady burst when first placed in the orchard. The manufacturer states that the dispenser was designed to perform in this manner. After its initial acclimation, the Isomate C+ dispenser released codlemone at a near steady-state rate from 0.5-0.8 mg day^{-1}. Our release results are in close agreement to the manufacturer's claim that the

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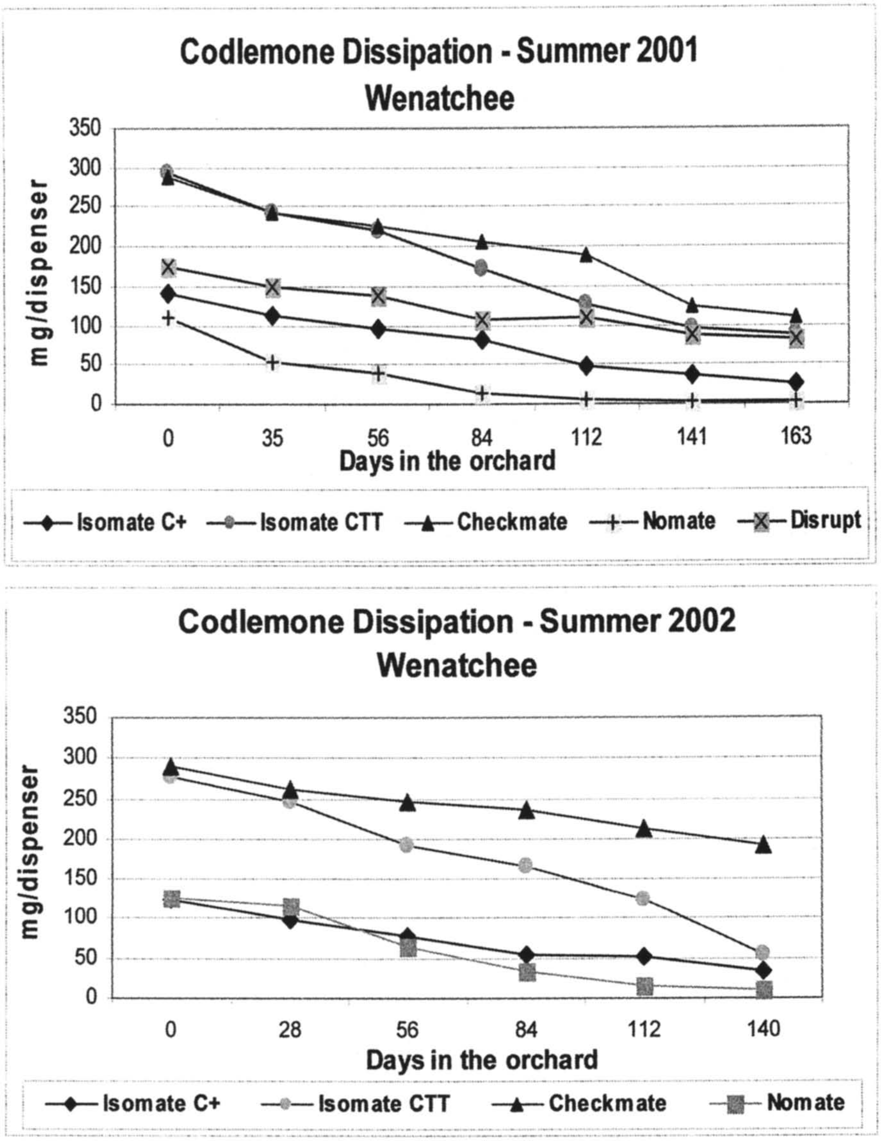

Figure 3. Year 2001 and 2002 residual analysis results from WSU-TFREC

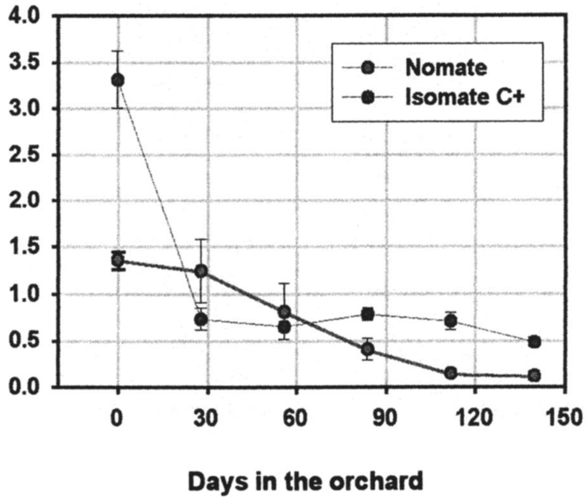

Figure 4. VT system evaluations of two dispenser types through 140 days of field aging. Each data point in this figure represents the average value of the four replicated dispensers sampled individually per interval date. Chemical release is expressed in milligrams codlemone per day (mg day^{-1}). The individual dispenser data was generated over a 2-hour collection period under constant temperature and airflow conditions.

individual dispenser release rate should approach 0.6 mg day^{-1}. Furthermore, the VT system release behavior closely matched anticipated total dispenser concentrations of field-aged dispensers taken from the same lot. Although the VT system data cannot be directly compared to residual dispenser evaluations, it does, however, give another measure of VT reliability in measuring codlemone release performance. VT results for the NoMate dispensers were also closely associated with anticipated total dispenser concentrations from field-aged dispensers taken from the same lot.

Variation of individual measurements in the VT system for field-aged dispensers from the same interval date was surprisingly low (Figure 4). Because of their relatively small size, dispensers like Isomate or NoMate are less likely to cause turbulent airflow within the VT chamber system. In the future, we will be providing release information on the more bulky larger membrane solid matrix dispensers such as Checkmate and Disrupt systems. The VT chamber system design must insure that laminar airflow contacts the surface of each dispenser type in a uniform manner. Otherwise, comparable emission data among the various dispenser sizes and shapes may not be attainable. Based on the results to date, the VT system is providing consistent and reproducible emission data for assessing release behavior of field-aged solid matrix dispensers.

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Conclusion

The 2001 and 2002 residual pheromone extraction data and 2002 VT system evaluations illustrated clear differences in chemical release among dispenser types exposed to identical orchard conditions. Some products released at a near constant rate and had residual pheromone after the end of the moth-mating season. From other dispenser products, codlemone was depleted rapidly or its release was attenuated presumably due to dispenser surface weathering. Residual pheromone analysis data for 2001 suggested that release from two of the dispenser products may not have been adequate to suppress CM mating during the second generation. However, this assertion is speculative because the amount of airborne codlemone needed to suppress codling moth populations below damaging levels within the orchard canopy with solid matrix dispensers has not been established.

The agreement in estimated codlemone emissions among different dispensers using two dissimilar laboratory approaches indicated further field research will be essential for optimizing dispenser performance. Areas of future research should also include direct ambient air evaluations of pheromone concentrations under actual canopy conditions in combination with field-aged residual and VT dispenser evaluations. These combined assessments will be very helpful in assessing the true efficacy of different dispenser types.

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Chapter 11

Evaluation of Catnip Oil as a Barrier to Termites

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The oil of catnip, *Nepeta cataria* (Lamiaceae), was tested in the laboratory as a soil-applied barrier to subterranean termites, *Reticulitermes virginicus* and *R. flavipes* (Isoptera: Rhinotermitidae). The essential oil consisted of two isomers of nepetalactone, the *E,Z*- and *Z,E*-isomers in a ratio of 36: 64. Tunneling was reduced at all doses in a vertical tunneling assay, and ceased completely at doses of 250 ppm and higher for *R. flavipes*. Horizontal tunneling through a treated sand barrier was reduced at all doses, eliminated at doses ≥ 250 ppm for *R. virginicus*, and reduced but not eliminated for *R. flavipes*. In both assays, mortality of neither species was high, indicating that the reduction in tunneling was due to repellency and not by attrition. The time to 50% dissipation (DT_{50}) of each isomer was dose dependent, ranging from 5.7 days at 100 ppm to 12.6 days at 1000 ppm for *E,Z*-nepetalactone, and from 7.7 days at 100 ppm to 18.6 days at 1000 ppm for *Z,E*-nepetalactone. We do not believe that catnip oil as presently tested would be an effective barrier against termites.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

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Termites (Isoptera) continue to be the most important and economically damaging pest of structures throughout the world, despite recent advances in termite control. Of the three major types of termites occurring in the United States (subterranean, drywood and dampwood), subterranean termites (Rhinotermitidae) are the most economically important. Subterranean termites are distributed throughout most of the contiguous 48 states plus Hawaii, and cause greater than $1.5 billion damage annually (*1*). This figure does not include the cost of prevention, repairs, loss of property value, or the costs of damage not directly attributable to termites (such as wind damage to termite-weakened structures). The heaviest infestations occur in the South. Drywood (Kalotermitidae) and dampwood (Hodotermitidae, Kalotermitidae) termites also cause significant damage in localized areas, mostly in Florida, the Gulf Coast and the West Coast, but are not as economically significant as subterranean termites.

Termite control for the last 70 years has relied mainly on soil application of termiticides. Termiticides are applied to the soil during construction, either on the ground before the slab is poured, or around piers, pillars and conventional foundations walls once they are in place. Treated wood is sometimes used in areas most vulnerable to termite attack, but is usually too costly to use throughout an entire structure. Termites may, in any case, move past treated wood to reach untreated wood (*2*). Post-construction preventive chemical treatments are more costly and complex to apply, and consist of trenching along the perimeter of the structure or drilling and rodding beneath the slab or along the perimeter, providing a continuous area of treated soil through which the termites must pass to enter the structure. Existing infestations are controlled by chemical treatments through trenching or rodding. If deprived of a moisture source, subterranean termites die in a relatively short time. Termiticidal baits have become popular in recent years. Typically, a monitoring station lacking the toxicant is placed near a building, and when termites are detected in the monitoring station the toxic bait is added. The termites then share the bait with nestmates, potentially spreading the toxicant throughout the colony. Baits are often used to control existing infestations, but may take months to have their full effect.

There are currently several active ingredients registered for soil application for control of termites: fipronil, imidacloprid, chlorfenapyr, and several pyrethroids, including permethrin, cypermethrin, bifenthrin and others. The Forest Service, US Department of Agriculture, Wood Products Insect Research Unit in Starkville, MS, USA conducts efficacy testing in support of soil-applied termiticide registration for the US Environmental Protection Agency (EPA). The Forest Service tests about three new formulations per year, with a new active ingredient being tested about once in every two years. Bait active ingredients include the insect growth regulators hexaflumuron, noviflumuron and diflubenzuron, and the metabolic poisons hydramethylnon and sulfluramid.

Although not new, the use of natural products for pest control is growing. Natural products are beginning to enter the homeowner and personal repellent specialty markets in the form of pyrethrin insecticides, derived from pyrethrum; avermectins and spinosad, derived from microbial products; azadirachtin, from the neem tree and the basis for several pesticides; and essential oils. Natural products, however, are not being evaluated to a large degree for termite control. Avermectin, thuringiensin, pine oil, limonene, rotenone and neem have been tested by the USDA Forest Service in the laboratory but not in the field, and registration of these products was not pursued. The toxic and repellent properties of several natural compounds to termites have been reported (*3* – *7*).

The essential oil of catnip, *Nepeta cataria* (Lamiaceae) is responsible for the well-known effect on house cats (*Felis domesticus*) and is a repellent of insects (*8*). Recently, it has been shown to be repellent to the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae) (*9*) and the German cockroach *Blattella germanica* (Blattodea: Blattellidae) (*10*). The catnip oil consists almost entirely of a mixture of two isomers of the monoterpenoid nepetalactone (*11*). The isomers (Figure 1) are designated as *E,Z*- and *Z,E*-nepetalactone.

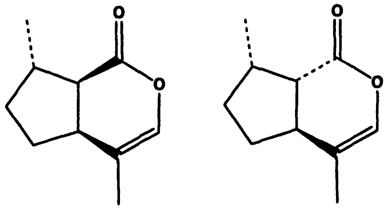

Figure 1. Structures of Z,E- and E,Z-nepetalactone, showing stereochemistry.

In this work, we review our experiments that have evaluated the potential of catnip essential oil as a barrier to subterranean termites in the laboratory (*12*). Both horizontal and vertical barriers were evaluated in laboratory-scale assays. We also measured the longevity of catnip oil in treated sand.

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Materials and Methods

Termites

Subterranean termites were collected from the Choctaw Wildlife Management Area of the Tombigbee National Forest near Ackerman, MS. Two populations, separated by 3 km, were collected from infested pine logs and taken to the laboratory for identification. Based on an examination of the soldiers (alates were not available), taxonomic keys (*13, 14*) were used to identify the populations as *Reticulitermes virginicus* (Banks) and *R. flavipes* (Kollar). Individual *R. virginicus* workers averaged 1.6 (± 0.01) mg, while *R. flavipes* were 3.3 (± 0.07) mg. Logs were kept in 30 gal (113.5 L) metal cans with lids, and termites were removed as needed throughout the test.

Essential Oil

Catnip essential oil was purchased from Kong Pet Products, Golden CO. The oil consisted of 36:64 *E,Z-*:*Z,E*-nepetalactone, determined by high performance liquid chromatography.

High Performance Liquid Chromatography (HPLC)

The oil was analyzed on a Waters 2695 liquid chromatography system, with a Waters Symmetry C_{18} column (4.6 × 75 mm, 3.5 µm pore size) (Waters Corporation, Milford, MA). A mobile phase of 60:40 methanol:water (isocratic) was used at a flow rate of 0.5 mL/min. Injection volume was 10 µL. A Waters 996 photodiode array detector was used for peak detection, scanning from 210 to 260 nm, with quantification of isomer peak area based on an external standard curve at 225 nm.

Vertical Barrier Assay

Sand treated with three doses of catnip oil (100, 250 and 500 ppm by weight) was selected for use in this test. One hundred g of sand was treated with catnip oil dissolved in acetone, and control treatments consisted of acetone alone. The sand was put on a jar roller for 5 min, the sand was removed from the jar and placed in a fume hood where the acetone evaporated at room

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temperature for 1 h. The tests were conducted in 2.5-cm diameter by 25-cm long glass test tubes (Figure 2). A southern yellow pine sapwood block, 2 x 1 x 1 cm, was placed at the bottom of each tube. Each tube was filled with a 50: 50 (v/v) mixture of sand and vermiculite substrate to a depth of 6 cm, and 5.5 ml of distilled water was added. Treated sand was added to a depth of 6 cm (about 35.5 g sand) on top of the sand-vermiculite mixture and 5.5 ml distilled water was added (about 15% moisture by weight). A top layer of sand and vermiculite substrate was added to the test tube to a depth of 6 cm and a 1.5-cm wooden cube was pressed 0.5 cm into the substrate. The top substrate was moistened with 5.5 ml of distilled water. Eighty worker termites plus one soldier were placed on top of the upper 6-cm substrate. Each tube was covered with a piece of Parafilm® (American National Can Company, Chicago, IL), and the tubes were placed in a darkened incubator at 25°C and 70% RH. After one week, the depth of visible tunneling in the treated sand was measured, the tubes were emptied and the number of termites recovered from each tube was recorded. The test was replicated five times for each concentration and termite species. The distance tunneled and the number of termites recovered were analyzed by ANOVA using SAS (*15*).

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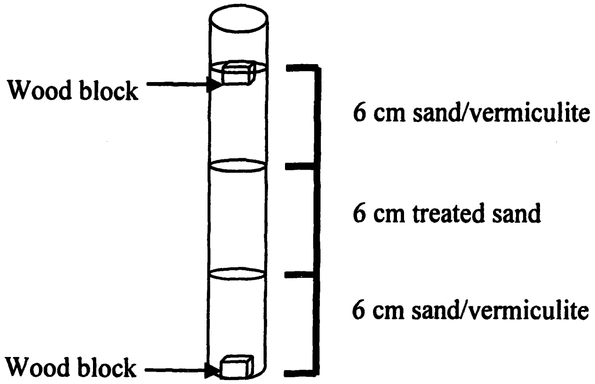

Figure 2. Vertical barrier assay to measure the depth of tunneling into treated sand.

Horizontal Barrier Assay

Sand treated with three doses of catnip oil (100, 250 and 500 ppm by weight) was selected for use in this test. The sand was prepared as described for the vertical barrier assay. Modifications of existing methods (*7, 16*) were used to determine the effect of catnip oil-treated sand on horizontal tunneling of termites. This method employed a zone of untreated sand, the "Introduction" zone, a "Barrier" zone of treated sand, and another untreated, or "Protected" zone, on the other side of the Introduction zone in a transparent 13.5 x 12.75-cm box (Figure 3). Paper cards were placed in the box to divide it into equal thirds. The sand, 100 g of the treated sand for the Barrier zone, 100 g untreated sand for the Introduction zone and 100 g of untreated sand for the Protected zone, were added to the boxes in a way to provide a barrier of treated sand through the middle of the box. The overall depth of the sand was about 1 cm. Each zone was moistened with 20 ml of distilled water (20% moisture by weight) and the paper cards were removed. One 2 x 1 x 1 cm block of southern yellow pine sapwood was placed in the sand in both the Introduction and Protected zones about 1.5 cm from the edge. A small amount of sand was excavated around the blocks to give the termites access to the bottom of the box and help prevent them from constructing shelter tubes over the top of the sand. Termites (200 workers plus 2 soldiers) were placed in the Introduction zone directly on the wood block. The boxes were covered with lids, sealed with Parafilm®, and then placed in an incubator at 25°C and 70% RH in the dark. The position of the Introduction zone, to the right or to the left, was randomized by using a random numbers table. Five replicates were conducted for each concentration and for the acetone control for both species. After seven days, the undersides of the boxes were photocopied to document the visible tunnels on the bottom of the box. Visible tunneling may not reflect total tunneling, because the termites may construct tunnels not visible from below. The photocopied tunnels and galleries were traced onto transparency film, photographed with a digital camera, converted to grayscale, and analyzed by using ImagePro (version 3.2, Media Cybernetics, Silver Spring, MD USA) for total visible area excavated by the termites. Surviving termites were extracted and counted from each of the three zones in each box (Introduction, Barrier and Protected zones). Percentage survival (total for the entire box) and percentage of area excavated were transformed by the arcsine of the percentage and analyzed by ANOVA (α=0.05). Percentage of termites surviving and percentage of area excavated from each zone were also arcsine transformed and analyzed individually by ANOVA (*12*).

Persistence of Nepetalactone Residues

Sand was treated as described above at doses of 10, 100, 500, 750 and 1000 ppm and analyzed by HPLC. A standard curve was constructed at identical conditions and the standard curve was used to determine the concentration of each isomer in the sand. Twenty g of sand were extracted with 20 ml methanol, filtered and injected into the HPLC for quantitation. This served as a check of nominal dose. The jars of sand were capped, sealed with Parafilm® and stored

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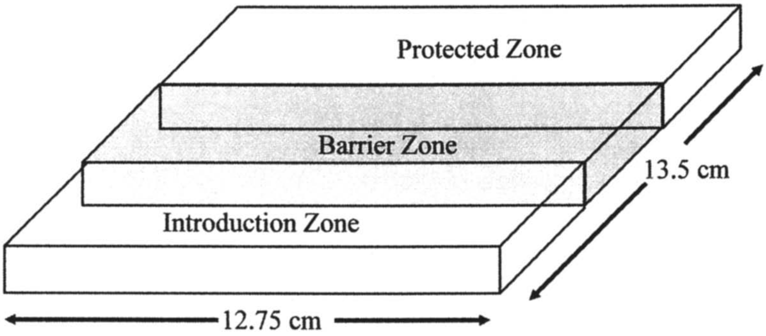

Figure 3. Layout of bioassay boxes for the horizontal barrier assay.

in an incubator at 25°C and 70% RH in the dark. At one-week intervals, 20 g portions of sand were removed from the jars, extracted with 20 ml methanol, and then analyzed by HPLC. Sampling continued until the treated sand was depleted. The data were used to determine the rate of dissipation of the individual nepetalactone isomers. The Proc Mixed function on SAS was used to determine significant effects for repeated measures (*15*).

Results and Discussion

Vertical Barrier Assay

Treatment of the sand with catnip oil had a dose-dependent effect on the depth of tunneling in the assay. The overall model for depth of tunneling related to dose and species was significant ($F = 31.22$; df = 7, 32; $P < 0.0001$). The depth of tunneling into the treated sand was significantly affected by catnip oil dose ($F = 72.80$; df = 3; $P < 0.0001$), but not by species ($F = 0.06$; df = 1; $P = 0.8119$) and the dose × species interaction was not significant ($F = 0.02$; df = 3; $P = 0.9963$).

The penetration into treated sand by the termites is presented in Figure 4. The average depth of tunneling decreased with an increase in dose. In the

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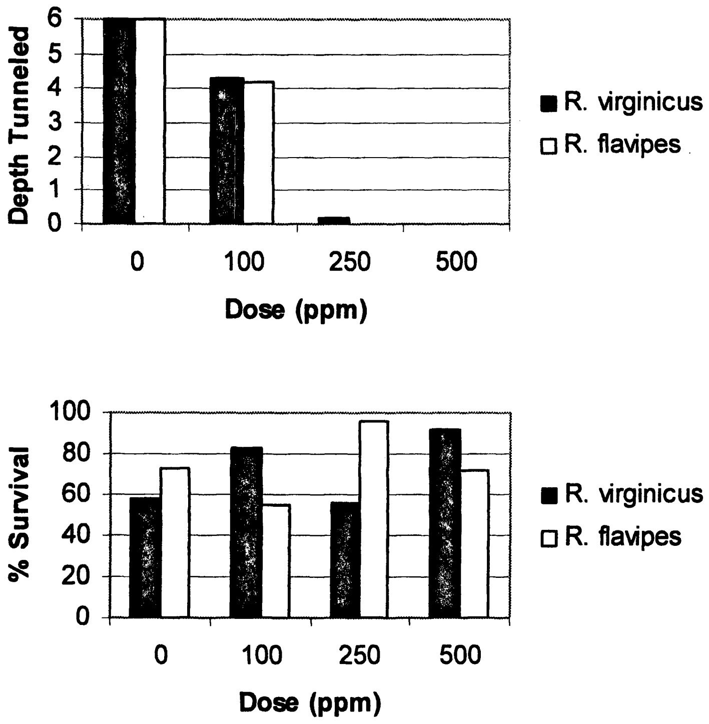

Figure 4. Top: Depth of tunneling (cm) into the treated zone of the vertical barrier assay. Bottom: Survival (7 days) of termites in the vertical barrier assay. Data modified from (12).

control, the sand was completely penetrated (tunneling measured 6 cm) by the termites in all five replications for both species. For *R. virginicus*, only one replication at 250 ppm had tunneling into the barrier (0.8 cm) and no tunneling was observed at 500 ppm. For *R. flavipes*, no tunneling was observed at 250 or 500 ppm. It is sometimes observed that penetrations are all-or-nothing, with penetrations to the full depth or no penetration at all. Such was not the case here. In these tests, intermediate tunneling depths were observed. Although the sand was completely penetrated in the controls for both species, at 100 ppm *R. virginicus* completely penetrated the treated barrier only twice, while *R. flavipes* did only three times at this dose. The percentage survival was not dose dependent ($F = 1.36$; df = 7, 32; $P = 0.2569$) (Figure 4). This indicates that the decrease in tunneling depth was not due to mortality. In some tubes, however, all termites were dead at the time of examination, probably due to infection with the bacteria *Serratia marcescens*; examined termites had the red heads characteristic of such infections. This happened once with *R. flavipes* in the control and twice at 100 ppm. The control tube with infected larvae was completely penetrated, while one 100 ppm tube with infected larvae showed no penetration and in the other the sand was penetrated to about one-half of its depth.

Horizontal Barrier Assay

Catnip oil acted as a horizontal barrier to *R. virginicus* and reduced tunneling of *R. flavipes*. The overall model for percentage of visible area excavated from the entire box (all three zones together) was significant (F = 27.56; df = 7,32; P <0.0001), and significance was seen for the factors dose (F = 49.01; df = 3; P <0.0001) and species (F = 39.75; df = 1; P <0.0001) The dose × species interaction was not significant (F = 2.04; df = 3; P = 0.1282).

For *R. virginicus*, the total area excavated from the entire boxes decreased from 16.4% in the control to 5.4% at 250 and 500 ppm (Figure 5). At these doses, excavation only occurred in the Introduction zone with no evidence of ecavation into the Barrier or Protected zones. The lack of recovery of termites from the Protected zone suggested that catnip oil acted as a complete barrier. However, in one case (the Barrier zone of the 500 ppm dose) a few termites were recovered from a zone in which no excavation was measured.

For *R. flavipes*, the total area excavated from the boxes declined from 25.7% in the control to 8.1% in the 500 ppm dose (Figure 5). On average, no zone at any catnip oil dose was completely free of excavation, although the Barrier and Protected zones of some individual replications were. Excavations from the Barrier and Protected zones were observed in three replicates at the 250 ppm dose and two replicates at the 500 ppm dose. In the Barrier and Protected zones, percentage of visible area excavated declined with increasing dose. Although tunneling was reduced, the oil could not be viewed as a “barrier” against *R. flavipes* in the strictest sense of the word.

The model for the survival of termites from the boxes was significant (F = 6.64; df = 7, 32; P < 0.0001), and significant effects due to dose (F = 10.84; df = 3; P <0.0001) and species (F = 8.80; df = 1; P = 0.0057) were observed. The total number of termites recovered from the boxes was significantly lower at the two highest doses, and fewer *R. virginicus* were recovered overall. This colony of *R. virginicus* seemed more susceptible than the *R. flavipes* colony to the toxic effects of catnip oil in the horizontal barrier test. Thus, the reduction in

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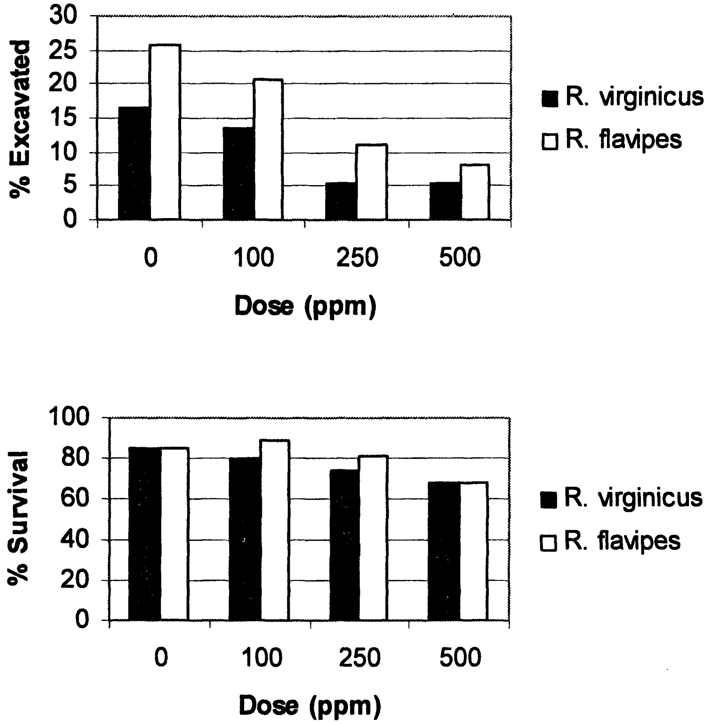

Figure 5. Top: Percentage of area excavated from the entire boxes in the horizontal barrier assay. Bottom: Survival (7 day) of termites in the horizontal barrier assay. Data modified from (12).

excavation was partly due to mortality, although repellent effects could not be ruled out.

In similar barrier tests, *R. santonensis* did not penetrate a barrier of soil treated with ~45,000 ppm isoborneol, but did penetrate some barriers treated with ~32,000 ppm. In the latter case penetration occurred along the edge of the test boxes. The termites did not penetrate a circular barrier of ~32,000 ppm isoborneol (*7*).

Our tests used catnip oil concentrations about 4 to 20 times above that of imidacloprid, which is applied as a termiticide in trenches at about 28 ppm. Fipronil is applied at about 26 ppm.

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Persistence of Nepetalactone

The sand was extracted and analyzed by HPLC after application of the catnip oil to determine the applied dose of each nepetalactone isomer. Nepetalactone was not detected in the controls, and nepetalactone recovered from the 10 ppm sand treatment was below the level of quantitation. All recovereies were less than the nominal applied doses, but recovery as a percentage of the applied dose increased with concentration. At 100 ppm, 31.1 and 38.0% of the nominal doses were recovered for *E,Z*- and *Z,E*-nepetalactone, respectively. Recovery increased to 67.7 and 71.9% for the 500 ppm dose, 79.5 and 85% for the 750 ppm dose, and 85.8 and 87.5% at the 1000 ppm dose (Figure 6).

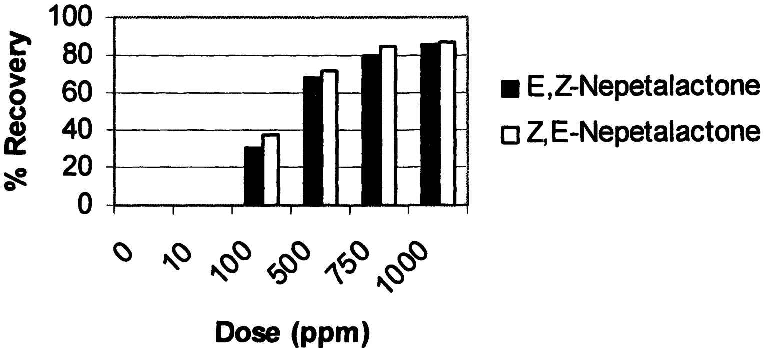

Figure 6. Percentage of isomer recovery at time = 0 days as a function of initial dose. Data modified from (12).

Dissipation of *E,Z*-nepetalactone was significantly influenced by dose ($F = 53.35$; df = 3; $P < 0.0001$), time ($F = 38.48$; df = 3; $P < 0.0001$), and the dose × time interaction ($F = 5.58$; df = 9; $P = 0.0006$). Dissipation of *Z,E*-nepetalactone was also significantly affected by dose ($F = 74.29$; df = 3; $P < 0.0001$), time ($F = 25.94$; df = 3; $P < 0.0001$), and the dose × time interaction ($F = 3.01$; df = 9; $P = 0.0179$). The dissipation of each isomer best fit a linear model at doses of 500 ppm and higher ($r^2 > 0.9327$ and 0.8876 for *E,Z*- and *Z,E*-nepetalactone, respectively, with higher r^2 values at the higher doses) (Figure 7).

The time to 50% dissipation (DT_{50}) of each isomer was calculated from the linear equations based on the dose recovered on the day of application. The DT_{50} for each compound was dose-dependent. *E,Z*-Nepetalactone had a DT_{50} of 5.7 d at 100 ppm, 10.9 d at 500 ppm, 12.5 d at 750 ppm and 12.6 d at 1000 ppm. DT_{50} values for *Z,E*-Nepetalactone were 7.7 d at 100 ppm, 15.4 d at 500 ppm,

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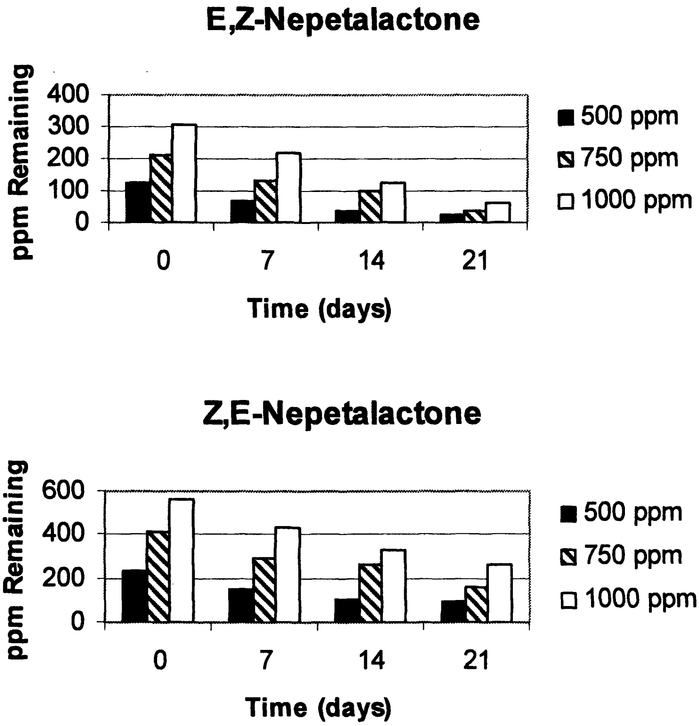

Figure 7. Dissipation over time of E,Z-nepetalactone and Z,E-nepetalactone. Data modified from (12).

17.2 d at 750 ppm, and 18.6 d at 1000 ppm. This increase in DT_{50} due to dose fit a logarithmic model with r^2 values of 0.9885 and 0.9998 for *E,Z*- and *Z,E*-nepetalactone, respectively (Figure 8). The DT_{50} values of *E,Z*-nepetalactone were ~68–74% that of *Z,E*-nepetalactone. The rate of dissipation (slope of the linear curve) was higher (more negative) for the *Z,E* isomer. This isomer degraded faster (in terms of ppm/d) but required a longer time to reach one-half of its initial recovered dose. Also, the rate of degradation was dependent on initial dose (Figure 8).

Nepetalactone dissipation characteristics are consistent with those observed for monoterpenoids by other researchers. Zhu et al. (*5*) found that monoterpenoids lost their effectiveness as repellents after about six days, but the sesquiterpenoid nootkatone was effective for >24 d. Based on microbial CO_2 emission, volatile plant oils and monoterpenoids were estimated to persist in the soil for greater than 15 days (*17*). Other natural products, such as azadirachtin A

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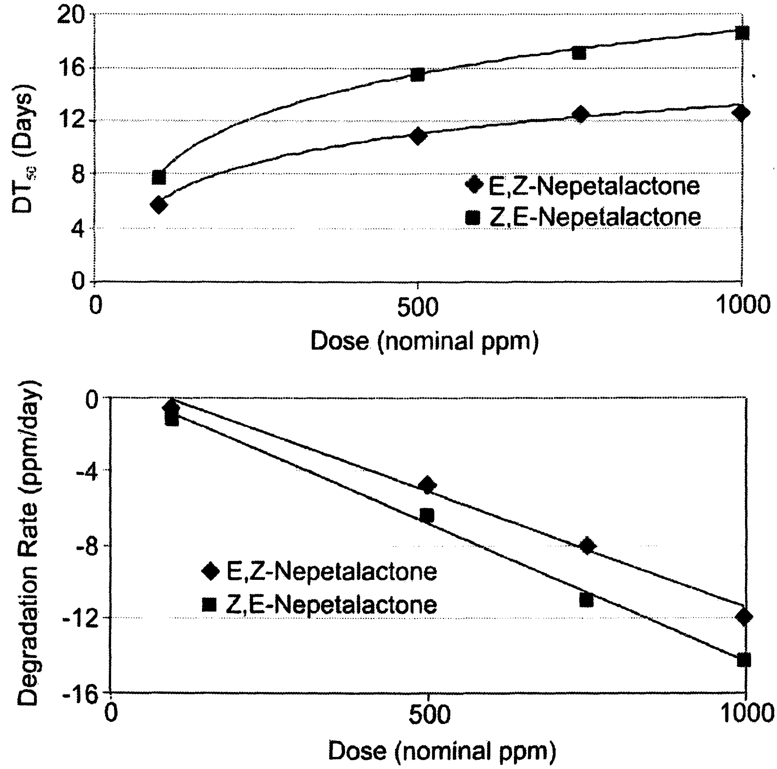

Figure 8. Top: DT_{50} values of each isomer as a function of initial dose. Bottom: dissipation rate (ppm/d) of each isomer as a function of initial dose. Data redrawn from (12).

had DT_{50} values in water of 25 to 29 days, and, similar to our study, the DT_{50} values were dose dependent (*18*). Formulated products dissipated more slowly than technical grade azadirachtin A. Persistence of the methyl ester of fusaric acid, a fungal toxin, ranged in half-life from 6.2 to 44.7 d, depending on temperature, soil moisture and soil type (*19*). Spinosad, a microbial natural product, had a soil half-life in the absence of light of 9 to 17 days (*20*) and a half-life in leaf litter from 2 to 12.4 days (*21*).

Of compounds used in termite control, chlorpyrifos had a half-life from ~30 to >720 d (*22*) or 315 to 462 d (*23*), depending upon dose, soil type and temperature. Imidacloprid had a half-life of 990 to 1230 days at termiticidal application rates (*23*). Fipronil had a half-life of 6 to 9 d, depending on soil type

(*24*), but no data exist for degradation at termiticidal rates. It seems reasonable that fipronil applied at rates for termite control will persist for much longer. Dose dependence of DT_{50} values may be due to an antimicrobial effect at high doses (*25*).

Catnip oil acts as a barrier to termite tunneling in laboratory assays. Few termites, if any, breached barriers of 250 ppm or higher. Avoidance behavior is likely responsible for the reduced tunneling, because mortality was not high in the behavioral tests. Some *R. flavipes* did breach the chemical treatment, so the term "barrier" is not appropriate for this species at these doses.

All these data taken together indicate that although catnip oil does slow or eliminate tunneling through treated sand, catnip oil as it is will be ineffective as a soil-applied termite chemical barrier. The soil concentration of the lowest effective dose is about 10 times that of the maximum labeled rate of imidacloprid or fipronil. At the current time, catnip oil is expensive, and use at effective rates would be cost-prohibitive. Longevity of the barrier is another limitation. The DT_{50} values determined here are 1/20 to 1/60 those of registered termiticides. The highest dose tested, 1000 ppm, would dissipate to below effective levels within 55 days. The US EPA requires that soil-applied termiticides remain effective in field tests for five years.

Some companies produce termiticide products for homeowner use with a "kills only" claim, which means that the product will kill an existing infestation, but will provide no protection against further attack. Most soil-applied termiticides are used in such a way to prevent attack, and our tests here were based on that intent. Catnip oil has good fumigant activity towards termites (*12*) and may be useful as a kills-only product. However, the proper tests have not been conducted to determine such. The oil may be useful in situations where isolated populations need to be destroyed with no expectation of residual control.

Chemical modification of the molecular structure into more persistent derivatives is another potential avenue for catnip oil development into a soil-applied termiticide, and for other compounds (e.g. monoterpenoids) of similar properties. This approach has been highly successful with the pyrethroid insecticides, based on the naturally-occurring but photo- and heat-labile pyrethrins. Lessening the reactivity of the side chains of the parent molecule has led to a vast array of pyrethroid insecticides. The neonicotinyl insecticides, based loosely on the structure of nicotine, are the result of analogous synthetic modifications that have led to the development of highly insecticidal products such as imidacloprid, thiocloprid, and nitenpyram.

One more potential avenue for increasing the efficacy of natural products would be through slow-release technology to increase longevity. Slow-release technology, however, often reduces the availability of the product and thus could increase the amount of essential oil required to provide protection. Perhaps an economical method for essential oil extraction would need to be

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developed first. Many other mint plants are cultivated for the food and flavor industry, and catnip should be amenable to such large-scale production. At the current time, however, the demand for catnip oil is quite low compared to other mint products (cat toys being the largest market). A deliberate effort on the part of mint producers would be required to supply the quantities needed for termiticide production. Only when these concerns are addressed would it be possible to determine economic feasibility of using catnip oil as a non-synthetic termiticide.

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Chapter 12

Natural Herbicides and Amendments for Organic Weed Control

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Various natural or non-synthetic herbicides and organic amendments that may be useful for weed control are generating intense interest among organic producers and gardeners. Among these products are corn gluten meal, wheat gluten, mustard seed meal, pelargonic acid, acetic acid (vinegar), and plant essential oil extracts (such as pine, clove, cinnamon, and thyme). This chapter reviews the literature with regard to efficacy of these products for pre- and postemergence weed control. Studies have focused on laboratory and field testing and indicate that some of these materials do have the potential for controlling weeds without harming the crop. However, the rates of application necessary to achieve acceptable control run in the hundreds to thousands of kg per ha, suggesting the commercial availability of non-synthetic products may be limited as certified organic production increases.

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

Natural or non-synthetic herbicides have recently garnered intense interest among farmers, gardeners, landscapers, and vegetation management personnel due to the perceived toxicity and potential for adverse environmental affects arising from the use of synthetic herbicides. This interest is particularly true among producers of organic fruits and vegetables and public grounds maintenance personnel, who find themselves with the daunting task of controlling weeds without the use of synthetic chemicals. In this chapter, several natural herbicides and organic amendments will be discussed, particularly regarding reports on their ability to control weeds.

Preemergence Products

In the context of this chapter, preemergence products are natural materials applied prior to emergence of weeds from the soil. These amendments contain compounds that, upon release into the soil, potentially control weeds by killing seed prior to, or immediately following, germination. Mulches differ in that they control weeds primarily by preventing light from reaching the soil surface, lowering weed seed germination and, if applied thickly enough, weed emergence. Preemergence products to be discussed include Brassicaceous seed meals, corn gluten meal, and wheat gluten.

Brassicaceous Seed Meals

Several species in the plant family Brassicaceae are grown for production of edible or industrial oil. After oil is extracted from the seed, the resultant meal contains 6 to 10% nitrogen (some 40% crude protein) and has been used for animal feed (*1*) and fertilizer (*2*). This defatted seed meal also may contain substantial quantities of compounds capable of pesticidal activity.

The principal pesticidal constituent of seed and leaf tissues of Brassicaceae species are several types of glucosinolates, which are organic anions containing a β-D-thioglucose moiety, a sulfonated oxime, and an aliphatic, aromatic, or heterocyclic side chain (*3, 4, 5, 6, 7*). In the presence of the endogenous enzyme myrosinase (β-thioglucoside glucohydrolase; EC 3.2.3.1), glucosides are hydrolyzed to form D-glucose, SO_4^{2-}, and several potential allelochemicals, depending on the aglycon chain structure and reaction conditions (*8, 9*). These metabolites include isothiocyanate (ITC), thiocyanates, nitriles, and ionic thiocyanate which, when present in sufficiently high concentrations, have been shown to reduce populations of nematodes (*10*), soilborne pathogens (*11, 12, 13, 14, 15, 16, 17*), and insects (*18, 19, 20*), and decrease germination of seeds and vegetative reproductive structures of plants (*21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31*). Glucosinolate production in Brassicaceous tissues may vary widely

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depending on growing conditions and cultivar/species (*32*), although ITC production is believed to be maximized by thorough mechanical or temperature-induced cellular rupture and mixing of myrosinase with glucosinolate in relatively wet soils (*33*).

There are few reports on the pesticidal effects of seed meal from Brassicaceous species as contrasted with green manure leaf/root residues. These reports generally show that seed meal does provide some control of soil pests, but effects are variable (*34, 35, 36, 37*). Plant/weed response to Brassicaceous seed meals in the field has also been inconsistent. High glucosinolate mustard seed meal (*Sinapis alba*, cv. 'IdaGold') banded over the row at 644 kg/ha after transplanting and again in spring reduced leaf area of strawberry (*Fragaria* x *ananassa*) transplants 16% but did not affect weeding time in the first iteration of a two-year trial (*38*). In the second iteration, strawberry leaf area was not affected by *S. alba* seed meal, but weeding time was reduced 16%. Application of low glucosinolate mustard seed meal (*S. alba*, mixed cultivars) at 644 kg/ha increased strawberry yield 14% and strawberry leaf area 16% in one of two years. High glucosinolate *S. alba* seed meal did not affect berry yield either year, and berry size was not affected by either *S. alba* seed meal treatment in either year.

First-year data from a different field study showed that high glucosinolate mustard seed meal (*Sinapis alba* cv. 'IdaGold') applied pre-plant incorporated (PPI) at 2240 kg/ha to a spinach (*Spinacia oleracea*) seed crop resulted in similar seedling biomass of shepherd's-purse (*Capsella bursa-pastoris*) as from incorporation of a preceding winter wheat (*Triticum aestivum*) cover crop (*39*). Mustard seed meal at that rate resulted in poorer shepherd's-purse control than incorporation of preceding mustard cover crops (*Brassica juncea* or *B. juncea/S. alba* blend) or treatment with metam sodium fumigant. No treatment in this study significantly affected common lambsquarters (*Chenopodium album*) seedling biomass.

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Corn Gluten Meal

Corn gluten meal (CGM) is the protein fraction of corn (*Zea mays*) grain extracted during the wet-milling process. CGM contains approximately 10% nitrogen (some 47% crude protein) and has been used for animal feed (*1*), and as both a fertilizer and weed control product (*40, 41, 42*). CGM breakdown products inhibit root formation during germination, resulting in weed seedlings that are less likely to survive water stress (*40*).

In greenhouse trials, CGM applied at equivalent rates to 324 g/m^2 reduced seedling survival of black nightshade (*Solanum nigrum*), common lambsquarters, creeping bentgrass (*Agrostis palustris*), curly dock (*Rumex crispus*), purslane (*Portulaca oleracea*), and redroot pigweed (*Amaranthus* *retroflexus*) by 75% or greater after 16 days (*43*). Reductions in root length of these species were greater than reductions in shoot growth (approximately 75% and 50%, respectively), and CGM incorporated into soil prior to seeding was generally more effective at slowing seedling growth than was a surface application at the same rate.

In field experiments, PPI treatments reduced weed cover compared to bare-soil plots in newly-transplanted strawberry (*42*). Powdered CGM applied PPI at rates from 100 to 400 g/m^2 gave reductions in weed cover ranging from 50 to 82% approximately 3 weeks after seeding 8 types of vegetable (*44*). Vegetable seedling survival in this study was also reduced by 48 to 83%, however, indicating that direct seeding into soil treated with CGM at these rates was not advisable. When used with sublethal dosages of pendimethalin in established turf, CGM applied at rates from 49 to 147 g/m^2 reduced the amount of herbicide required to provide 75 to 85% control of large crabgrass (*Digitaria sanguinalis*) from 88 to 29 mg ai/m^2 (*45*). CGM banded over strawberry rows at 487 kg/ha after transplanting and again in spring increased weeding time 14% but increased the number of strawberry daughter plants produced (*38*). In the second iteration, both 487 and 974 kg/ha CGM applied twice increased number of runners and daughter plants while the lower rate also increased strawberry leaf area, but neither rate significantly reduced weeding time. Berry yield was increased 17% by the high rate of CGM in one of two years, but neither rate influenced berry size in either year.

In an effort to concentrate the active ingredient of CGM and thereby reduce the amount of CGM product necessary to apply to adequately control weeds, alternative formulations of CGM were investigated (*46*). In this study, CGM and eleven processed CGM samples were tested for herbicidal activity on smooth crabgrass (*Digitaria ischaemum*), creeping bentgrass, and/or perennial ryegrass (*Lolium perenne*). Of all processed CGM materials, the bacterial proteinase gluten hydrolysate was the most active, reducing grass seed germination in petri dishes by 90% or more at rates from 0.56 to 1.18 g/m^2 (raw CGM reduced germination to the same level at 34.4 g/m^2). In addition to improving the activity of CGM, this hydrolyzation process also rendered the water insoluble meal a water soluble product that could be applied as a liquid through conventional herbicide application equipment. The root-inhibiting compound in corn gluten hydrolysate (CGH) was isolated and identified as a blend of five dipeptides (*47*). The dipeptides alaninyl-alanine and glycinyl-alanine were the most inhibitory of perennial ryegrass seed germination, followed by alaninyl-glutamine, alaninyl-asparagine, and glutaminyl-glutamine. Subsequent research indicated that root tip cells treated with alaninyl-alanine did not produce nuclei and other cellular components and displayed structural weakening of cell walls, resulting in breakage and loss of cytoplasmic integrity (*48*).

Field testing of CGH and CGM was conducted in matted-row strawberry (*49*). CGH reduced dicot weed number in only one of four years, a 59% reduction at the highest rate (29.3 g/m^2). CGM applied at an equivalent rate of

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nitrogen as for CGH reduced dicot weed numbers in two of four years (40 and 49% reductions), although in one year the CGM application also increased weed cover from 3% in untreated plots to 7%.

Wheat Gluten

Wheat has been identified as producing compounds allelopathic to other plant species in straw or in seedling root exudates (*50, 51, 52*). While growth of weeds mulched with wheat straw may be reduced by allelopathic leachates, growth is also inhibited by changes in temperature, moisture, and light resulting from the mulch itself, making actual allelopathic effects difficult to measure (*53*). Discussion of "mulch effects" and seedling exudates, however, are beyond the scope of this chapter on organic amendments for weed control.

Over half a century ago, water extracts from wheat seed coats and fruits were shown to inhibit germination and growth of several plant species (*54, 55*). More recently, wheat gluten (WG) has been reported to inhibit weed seedling growth (*56, 46*). The two major proteins in WG, gliadin and glutenin, become highly elastic when mixed with water, trapping the CO_2 released from yeast fermentation in dough and causing bread to rise (*57*). Depending on the hardness of the wheat that is used, flour may range from 8 to 15% N, making WG a potential fertilizer.

In greenhouse trials, WG applied at 10 g/m^2 reduced by ≥75% after 16 days the root length of annual ryegrass (*Lolium multiflorum*), curly dock, quackgrass (*Elymus repens*), leafy spurge (*Euphorbia esula*), redroot pigweed, and orchardgrass (*Dactylis glomerata*) seedlings. Similarly 30 g/m^2 reduced root length of spotted knapweed (*Centaurea maculosa*), annual bluegrass (*Poa annua*), Canada thistle (*Cirsium arvense*), and bean (*Phaseolus vulgaris*) seedlings (*56*). As noted with CGM, shoot growth was not inhibited as much as root growth in these trials. In separate studies, WG at 2.78 g/m^2 reduced perennial ryegrass seed germination 56%, while hydrolyzed WG at the same rate reduced germination 94% (*46*).

In field experiments, WG banded over strawberry rows at 700 kg/ha after transplanting and again in spring increased yield 14% but did not influence strawberry or weed growth (*38*). In the second iteration, weeding time was increased 9% after treatment with WG while strawberry growth and yield were not affected.

Postemergence Products

Postemergence products are defined in this chapter as natural or non-synthetic materials applied to weed foliage after emergence from the soil. In

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general, natural postemergence products display a direct effect on leaf epidermal cells, resulting in loss of cellular integrity, followed by tissue desiccation and, provided damage is severe enough, seedling mortality (*58, 59*). Given this mode of action, these products are nonselective, causing injury to most foliage with which they come into contact. Consequently, if postemergence products are used in organic vegetable or fruit production, care must be taken during application to prevent over-spraying foliage of desirable vegetation.

Acetic Acid

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Acetic acid is the compound in vinegar that most likely accounts for any postemergence control of treated weeds. The distilled white and cider vinegars commonly available in grocery stores contain approximately 5% acetic acid in water. Formulations with higher acetic acid (or acetic + citric acid) concentrations (up to 25%) are available as herbicides. Published studies regarding the use of acetic acid as a herbicide are few; of these, many are found as internet articles. One such study found that acetic acid in concentrations from 5 to 20% provided 80 to 100% control of giant foxtail (*Setaria faberi*), common lambsquarters, smooth pigweed (*Amaranthus hybridus*), and velvetleaf (*Abutilon theophrasti*) seedlings from 3 to 9 inches tall (*60*). Top-kill of Canada thistle in this study using 5% acetic acid was also reported. Vinegar has been used with some success to control liverwort (*Marchantia polymorpha*) and silver thread moss (*Bryum argenteum*) growing in ornamental plant containers (*61*). Acetic acid at 2.5 to 5% applied as a drench to dry irrigation canals have also inhibited tuber sprouting of hydrilla (*Hydrilla verticilata*) by 80 to 100% (*62*). In turf, one or three applications of 20 to 25% acetic acid in August resulted in 82 to 99% control of crabgrass, ground ivy (*Glechoma hederacea*), and broadleaf plantain (*Plantago major*) at 5 weeks after treatment (*63*).

In greenhouse trials, it was demonstrated that while 20 to 30 L/ha glacial acetic acid averaged over three concentrations (5, 10, and 20%) provided >90% control of Indian mustard (*Brassica juncea*), the same rate caused <5% injury to oat (*Avena sativa*), indicating that acetic acid could potentially provide selective broadleaf weed control in cereals (*64*). A field trial using vinegar (10% acetic acid) prior to seeding spring wheat (cv. 'Eatonia') gave >80% control of shepherd's-purse when applied at volumes of 1600 L/ha or higher (*65*). Wild mustard (*Sinapis arvensis*) and cowcockle (*Vaccaria hispanica*) were controlled >80% when vinegar was applied at volumes of 800 L/ha or higher, although wheat was injured at volumes >400 L/ha. Application volumes of 400 to 800 L/ha resulted in wheat yields better or similar to yields following a combination of pre-seeding treatment with glyphosate and postemergence application with bromoxynil + MCPA.

Essential Oils

Essential oils have been defined as natural plant products containing flavors and fragrances that provide characteristic odors (*66*). Several essential oils have been shown to exert substantial biological activity on pest organisms (*67*). Essential oils of cinnamon (*Cinnamomum zeylanicum*), clove (*Syzgium aromaticum*), savory (*Satureja montana*), summer savory (*Satureja hortensis*), rosemary (*Rosmarinus officinalis*), and thyme (*Thymus vulgaris*) have been shown to inhibit growth of microorganisms such as *Aspergillis*, *Botrytis*, *Camplobacter*, *Clostridium*, *E. coli*, *Fusarium*, *Listeria*, and *Salmonella* in foods or stored grain (*68, 69, 70, 71, 72*). Essential oils have also been tested as insect and mite repellents (*73, 74, 75, 76*), fish anaesthetics (*77, 78, 79, 80*), and antioxidants (*81, 82, 83*). Essential oils can affect germination of seeds (*84, 85, 86, 87, 88, 89*) and inhibit sprouting of potato (*Solanum tuberosum*) tubers (*90*).

Although anecdotal reports of the use of essential oils as herbicides are relatively common, very few reports have been published. In greenhouse experiments, dandelion (*Taraxacum officinale*) leaf cell membrane permeability was increased by applications of 1 to 2% concentrations of essential oils from cinnamon, clove, summer savory, and thyme (*91*). These same essential oils caused injury to johnsongrass (*Sorghum halepense*), common lambsquarters, and common ragweed (*Ambrosia artemisiifolia*) when applied at 1% and killed these weeds at concentrations of 5 to 10%. Pine oil at 5 to 17% applied to Indian mustard and oat selectively controlled the mustard only at volumes of 50 L/ha or less (*64*).

The major chemical constituents of several essential oils have been identified. Eugenol was found to be a primary constituent of cinnamon oil (84%) and was shown to possess postemergence herbicidal activity (*91*). Another prominent constituent of some cinnamon oils is *trans*-cinnamaldehyde, constituting 81% of cinnamon cassia oil and 62% of cinnamon bark extract (*69*). Eugenol is also the primary constituent of clove oil, constituting some 70% of clove bud extract and 79% of leaf extract (*92*). Major terpenoids of pine oil extracted from four species of *Pinus* were α- and β-pinene, myrcene, and limonene, although each accounted for <20% of the oil constituents (*75*). The major constituents of summer savory oil were found to be γ-terpinene and carvacrol (41 and 39%, respectively)(*93*). The primary constituents of thyme oil were identified as thymol, linalool, and carvacrol (37, 9, and 5%, respectively)(*69*) while others (*84*) reported thyme oil as containing 44% thymol.

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Pelargonic Acid

Pelargonic acid is a naturally-occurring fatty acid having substantial herbicidal activity (*58, 94, 95*). It displays fungicidal and insecticidal activity, but those uses are limited by its high degree of phtytotoxicity (*59*). Pelargonic acid has also been shown to aid in control of liverwort and silver thread moss in greenhouse containers (*61*). In greenhouse trials, pelargonic acid applied to Indian mustard and oat was not selective at 3 or 6%, and was more active than either 5 to 20% acetic acid or 5 to 17% pine oil on the same two species (*64*).

Pelargonic acid (at 0.5 to 3%) mixed with glyphosate or glufosinate did not improve herbicide effectiveness on annual or perennial weeds in the greenhouse (*96, 97*). When mixed with glyphosate or glufosinate and applied to herbicide-resistant soybean (*Glycine max*) in the field, pelargonic acid improved yellow nutsedge (*Cyperus esculentus*) control, but only at 6 days after treatment; soybean growth and yield were not significantly affected by these applications.

Conclusions

Various non-synthetic products derived from plants have been tested for their efficacy in weed control. Both preemergence and postemergence applications were studied. Brassicaceous seed meals, corn meal gluten, and wheat gluten have potential for selective control of weeds following preemergence application. In addition to testing whole materials, some studies have attempted isolation of an "active ingredient" to specifically test its bioactivity. In addition to weed control, processed plant materials may also have an impact on crop productivity by augmenting nitrogen in the soil.

Materials suitable for postemergence application include acetic acid, various essential oils, and pelargonic acid. Owing to a generalized mode of injury with these materials, selectivity will be hard to achieve so precaution must be used in their application to avoid exposure of the crop.

At this point in the testing of non-synthetic products that would have utility for control of weeds under certified organic production practices, it is clear that potentially efficacious materials useful as preemergence herbicides must be applied at rates of hundreds to thousands of kg per ha. By comparison, modern synthetic herbicides are used at rates of tens of g to a few kg per ha. As organic production practices continue to be adopted, availability of the large quantities of suitable materials needed for adequate weed control could become a limiting factor to their use.

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Chapter 13

Inducible Plant Defenses: Prospects for Disease and Stress Control

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One approach to improve disease resistance in plants and reduce pesticide usage is to take advantage of the plant's inducible defenses. These defenses can be activated by spraying with compounds such as salicylic acid and are environmentally safe means of disease control. As discussed in this chapter, although there are numerous examples of inducible defenses being used commercially for disease control and environmental stresses, much still has to be learned in order to most effectively use this technology.

There are multiple methods used to manage plant diseases. In addition to good cultural practices, primary approaches for plant disease control include the use of resistant plants, which is the most desirable method, as it requires minimal input from growers. Limitations to this approach are that sources of resistance may not be available or resistance breaking down over a period of years. New sources of resistance can often be introduced by breeding, but this process can take over a decade to produce a new variety. Transgenic approaches can generate resistant plants more quickly, although use of this technology is precluded in many markets at this time. Pesticides can be a very effective means of disease control, but in addition to being costly, their use may raise environmental and health concerns among the public.

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A newer option for disease management is to manipulate the plant to induce defenses that can enhance its resistance to disease and even environmental stresses. Some plant defenses are constitutive such as the leaf cuticle or trichomes that deter many pathogens or pests (*1*), and compounds such as phytoanticipins that have antimicrobial effects (*2*,*3*). Other plant defenses are inducible, typically not activated until triggered by pathogens, stress or chemical signals. Numerous excellent reviews are available that focus on basic aspects of plant inducible defenses (4,5). This chapter will instead focus on more applied aspects of inducible plant defense mechanisms and examine some unresolved questions that will impact how best to use this technology for maximal success in the field.

Plants have evolved diverse mechanisms to resist disease and environmental stress. Interestingly, in some cases plants may be susceptible to certain biotic or abiotic stresses not because they lack mechanisms to resist such stresses, but because the plant does not activate its inducible defense mechanisms in a sufficient and timely manner. Conceptually, this is an important point, emphasizing that in some instances crops don't necessarily need additional genes to be introduced in order to give a desired response. While new varieties with improved disease resistance can be developed if a source of resistance is available, even in a best-case scenario this takes years. Numerous cultivars currently in widespread use have many agronomically desirable traits but poor disease resistance. However, utilizing induced resistance (IR) may extend the usefulness of those existing varieties with shortcomings primarily related to disease susceptibility.

Compounds are now available that activate inducible defenses when sprayed on plants. Over ten companies market products purported to activate inducible defenses such as Systemic Acquired Resistance (SAR) for disease control in the field. IR is an environmentally safe method of disease control. The compounds used to treat the plants do not act directly upon the pathogen, but rather activate plant defenses. Some of the advantages of using IR are:

- Safer to apply with minimal risk to workers.
- Environmentally safe. IR activators are readily degraded in plants and soil. One SAR product (Messenger) was awarded the EPA's "Presidential Green Chemistry Challenge Award."
- One of the best options for the organic industry, which has a limited number of pesticide choices.
- Can confer enhanced, long lasting resistance to a broad range of pathogens and environmental stresses.
- One of the few methods proven to be effective against viruses directly, as opposed to the virus vector.

- Works on existing cultivars.
- Resistance is due to synergistic effects of many genes and thus is likely to be more durable than single gene based resistance strategies.

Induced Resistance and Plant Activators

IR has been an intensely researched field for decades at the basic science level, but is a relatively new technology for commercial disease control. Many growers are just now being exposed to this technology and reactions to the concept can range from excited to skeptical. In the commercial and consumer realm, products eliciting IR are generally referred to as "SARs" or "plant activators." Moving this technology from the lab to the field is perhaps proving to be more complicated than initially anticipated. It should not be assumed that all crops will respond identically to treatment with plant activators. For example, potato has significant differences in salicylic acid (SA)-mediated signaling from the tobacco and *Arabidopsis* model systems (6). Successful use of IR will likely require customization for each crop and perhaps even each pathogen. In many instances, the molecular biologists and biochemists involved in the basic research are not involved in defining the optimal methods to use this technology in the field. These researchers may have minimal involvement in field trials, which can be much more complicated than limited testing done in growth chambers or greenhouses. Field trials with plant activators often involve only spraying one or two different concentrations of the compound being tested and then evaluating differences compared to untreated plants. For various reasons, this is not necessarily the ideal approach to optimize the effect of plant activators and usually no attempt is made to determine if or how well the expected defense genes were induced.

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Basic Aspects of Plant Inducible Defenses

Induced Systemic Resistance (ISR) is one type of IR and jasmonate and ethylene are signal molecules that are important regulators of this response in plants. Ethylene and jasmonate are also involved in wounding responses that can confer resistance to insects or cause the plant to produce volatiles that attract herbivore predators or parasites.

SAR, another type of IR, is by far the best characterized inducible defense response and SA is used by plants as a key SAR mediator. These plant defenses are regulated by complex signal transduction chains that are increasingly well defined and thus increasingly amenable to manipulation using pharmacological and biotechnological approaches. Jasmonic acid, ethylene, SA and nitric oxide cause substantial alterations in gene expression and have complex crosstalk (*7,8*).

Compounds that activate SAR are comprised of both natural and synthetic compounds, some of which are shown in Figure 1. Although many of these compounds are structurally unrelated to SA, they are functional analogues of SA.

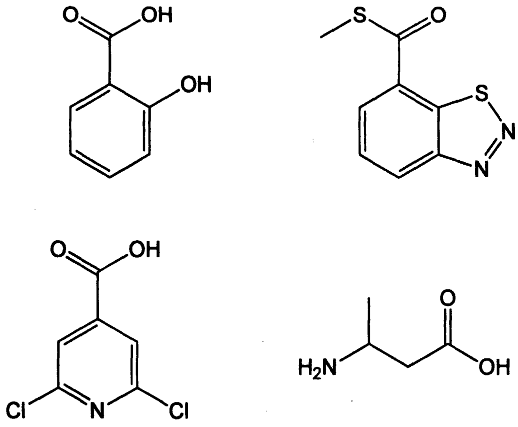

Figure 1. Structures of four compounds that activate SAR. Clockwise, starting with the top left, they are salicylic acid; 1,2,3-benzothiadiazole-7-carbothioic acid, S-methyl ester (BTH); DL-β-aminobutyric Acid (BABA); and 2,6-dichloroisonicotinic acid (INA).

Spraying these compounds on plants will induce SAR. In nature SAR is activated after challenge with certain pathogens. Upon encountering a pathogen, plants respond with substantial changes in gene expression and can activate inducible defenses that confer enhanced disease resistance that lasts for weeks to months. Plants expressing SAR become more resistant to subsequent infection from not only the original pathogen but also from a wide range of pathogens (*6*). For example, if a tobacco leaf of a variety carrying the N gene is infected with Tobacco Mosiac Virus (TMV), necrotic lesions develop on the infected leaf and effectively restrict the spread of the virus. Concurrent with the restriction of virus spread, various responses occur in the infected leaf tissue including the production of pathogenesis-related (PR) proteins. Then, a couple of days later, a signal moves out of the infected leaf and induces multiple defense responses in uninfected parts of the plant, at which point the entire plant is now manifesting SAR. Salicylic acid (SA) levels increase throughout the plant after infection and are necessary for SAR to develop (9). Spraying a tobacco plant with salicylic

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acid induces substantially the same set of genes as does infection with TMV. In addition to its role in SAR, SA is involved in local defenses and R-gene mediated resistance (6). Exactly how SAR results in enhanced resistance is not known, but many PR-proteins expressed during SAR have been shown to be antagonistic to pathogens. Some of the PR-proteins expressed during SAR and their functions are listed in Table 1 (10).

Table I. Pathogenesis-Related Proteins (PR) and Related Functions

Protein Family	*Function*
PR-1	Inhibitory towards some *Oomycetes*
PR-2	β-1,3-glucanases
PR-3	Chitinases
PR-4	Antifungal, chitin binding
PR-5	Antifungal; may disrupt fungal membranes
PR-6	Protease inhibitors
PR-7	Endoproteases
PR-8	Class III chitinases, chitinase/lysozyme
PR-9	Lignin-forming peroxidases, peroxidase-like proteins
PR-10	Ribonucleases, Bet v 1-related proteins
PR-11	Class V chitinase endochitinase activity
PR-12	Plant defensins
PR-13	Thionins
PR-14	Nonspecific lipid transfer proteins

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SA-Mediated Signaling in Potato

We are interested in exploiting IR for potato disease control in the field. As an initial step towards this goal, we are characterizing some basic features of inducible defenses in potato and have observed notable differences relative to the more studied model systems. SA levels are usually very low in plants, only increasing after an infection. This increase in SA is necessary to trigger SAR. However, potato has very high basal levels of SA (11,12). Potato leaves were measured that had total SA concentrations of 5,000 ng/g fresh weight, which is about a hundred-fold higher than the basal levels in tobacco. This observation raised questions about whether potato has some level of SAR in effect constitutively, or conversely, whether the high SA levels contribute to making potato less responsive to SA. Subsequently, we found that potato can be very responsive to SA and that concentrations as little as 250 uM induce PR-1 (12). Curiously, potatoes grown in the field appeared to be less competent to respond to BTH later in the growing season as judged by PR-1 induction (12). If this observation is found to be widespread among plants it will complicate the use of SAR in the field because it suggests plants may not always be equally responsive to chemical induction of SAR.

Another unexpected finding with field grown potatoes was that PR-1 expression increased later in the growing season in untreated plants that had no visible signs of disease (12). One interpretation of this observation is that potato spontaneously manifests SAR during the course of a growing season. Findings such as these illustrate the complexities of using IR in field conditions as opposed to tightly controlled laboratory conditions. In nature, multiple environmental cues including pathogens, insects, temperature, and drought stress may have complex consequences, activating multiple defense/stress signaling pathways that may have synergistic or antagonistic effects upon one another.

Additional differences in potato include a higher basal level of PR gene expression (13) and a hypersensitivity to BTH (Figure 1) under certain conditions (12). These differences caution against assumptions that different crops will respond identically to the same plant activator.

Most research on SAR has focused on leaves; consequently less is known about SAR in roots. We observed that treating leaves with BTH or the protein harpin strongly induced chitinase expression in roots (Figure 2), suggesting an enhanced ability to resist fungal pathogens. Furthermore, SA was shown to have a role in resistance of tomato to root-knot nematode (*14*). Thus, it seems likely that SA is important not only in foliar defenses, but also in roots.

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Issues to be Resolved for Optimal IR Usage

Because IR activators represent a new technology, growers can be confused about how IR fits into their integrated disease management program. Are chemical activators pesticides, do they replace pesticides or are they a new and additional step in disease management?

In most cases it seems improbable that IR will give complete protection against a pathogen. A more likely scenario is that plant activators will enhance resistance, but not give immunity. Therefore it is unlikely that plant activators will replace traditional pesticides. If this turns out to be the case, then how best to use plant activators and pesticides together in a program? Might the plant activators enable a reduction in the amount of pesticide needed to be applied? For some diseases, enhanced resistance may be good enough, while for other pathogens there may be zero tolerance. This likely needs to be determined crop-by-crop and pathogen-by-pathogen. On the other hand, there may be a few pathogens that can be completely controlled by IR and again this needs to be determined on a case by case basis.

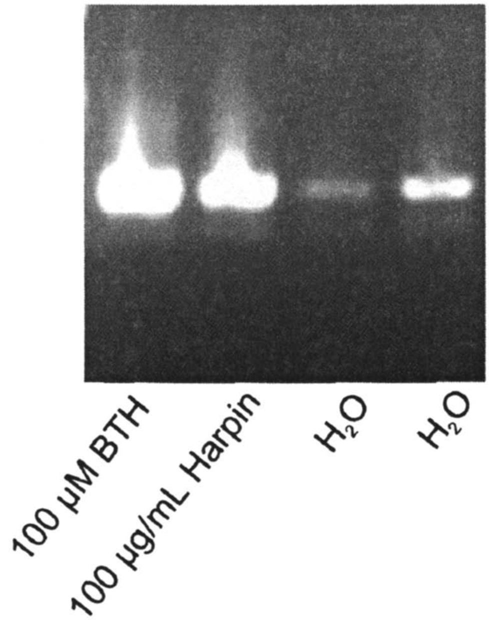

Figure 2. Agarose gel loaded with equal amounts of rt-PCR reactions. RNA was isolated from potato roots 24 hours after being sprayed with the indicated treatment and amplified using chitinase primers.

Also needing to be more clearly defined is whether activating induced resistance has a significant effect on yield. Once again, this answer may vary from one crop to another. Not yet clear is how long protection lasts after a treatment and what is the appropriate growth stage when plants should be treated. Likewise, during the course of a growing season, do plants always remain equally responsive to the plant activators? With potato there are some indications that plants are not always equally competent to respond to the triggering signals at all growth stages (12). Furthermore, can different types of IR be used together, for example, SA- and jasmonate-mediated pathways or are they mutually antagonistic? If they are mutually antagonistic, does that mean that activating SA mediated defense mechanisms in plants will suppress jasmonate signaling and perhaps result in the plant being more susceptible to the pathogens or insects that jasmonate-mediated defenses are effective against? Clearly, despite an abundance of ongoing research into IR many questions remain unresolved.

To accurately assess the effectiveness of IR as a disease management option, its efficacy must be evaluated under field conditions. Plant molecular biologists focusing on disease resistance under greenhouse conditions ultimately must relate resistance to what happens in nature. Resistance observed in the lab,

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but not in nature, is not real disease resistance. Confoundingly, a plant might be susceptible to a pathogen in the lab, but resistant in the field. Assessing plant resistance in the field or lab can present a conundrum. Ideally, a resistance assay done in the lab should mimic what the plant would encounter in nature, as inoculating with an artifically high density of pathogen in a resistance assay can overwhelm potentially effective plant defenses. Conversely, evaluating the efficacy of IR in the field is complicated by wild variations in disease pressure that can vary from season to season and field to field. A carefully planned experiment may be evaluated over the course of a growing season but yield no useful information because little or no disease was present in treated or untreated plants. Other questions still awaiting resolution in field trials include whether a given treatment might be effective in a location where disease pressure is modest, but not where disease pressure is severe. A fundamental question is whether different varieties of a given crop will exhibit similarly effective induced resistance or whether some varieties will be substantially superior for use in disease management programs that wish to utilize IR.

Additional Applications of Induced Defenses

Inducible defenses may prove to be effective against other stresses besides pathogens. SA was shown to reduce the toxicity of cadmium in barley (*15*), increase tolerance to chilling in maize (*16*), and increase general stress tolerance in beans and tomatoes (*17*). Findings such as these suggest a plethora of beneficial applications of manipulating plant signaling remain to be discovered. As the signal transduction pathways regulating inducible defenses become more defined, it is probable that increasingly effective methods of chemically manipulating the pathways will lead to new methods of biotic and abiotic stress management in plants.

Acknowledgments

BTH, trade name Actigard, was a gift of Syngenta. Harpin was supplied as Messenger, a gift of Eden Bioscience. The USDA-ARS does not endorse any products.

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Chapter 14

Messenger®: An Environmentally Sound Solution for Crop Production and Protection

Zhongmin Wei and Frederick S. Betz

**Eden Bioscience Corporation, 3830 Monte Villa Parkway,
Bothell, WA 98021-6942**

Messenger® is a novel product that enhances yield, quality, and disease and pest resistance in treated plants. These enhancements are based on the activity of a new class of nontoxic, naturally occurring proteins called harpins that are the active ingredients in Messenger. Harpin proteins activate certain plant growth and reproductive systems and trigger natural defense systems in plants that protect nonspecifically against many diseases and pests. When applied to crops, harpin increases plant biomass, photosynthesis, nutrient uptake and root development that ultimately leads to better crop quality and yield increases of 10 to 20%. After application, harpin-induced activity initially results in increased ion exchange through cell membranes that is followed by substantial changes in the level of gene expression. Up or down-regulated genes are generally associated with signal transduction pathways related to specific functions including protein and sugar transport, cell growth, plant development, flower induction, fruit set, and disease, pest, and stress resistance. These harpin-induced responses are initiated through a binding process between harpin and HrBP1, a putative harpin receptor protein that exists in all crops tested thus far. Using environmentally friendly fermentation and formulation processes, Eden Bioscience has advanced harpin technology to the marketplace in the form of Messenger, the first commercial product of this new crop management technology. EPA approved Messenger in April 2000 as a biochemical pesticide for yield enhancement and disease management in more than 40 crops.

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

> Messenger is virtually non-toxic, does not leach, bioacccumulate, or persist in the environment, and leaves no detectable residues on treated crops. EPA officially recognized the potential benefits of Messenger with its Presidential Green Chemistry Award in 2001: "Messenger: A Green Chemistry Revolution in Plant Production and Food Safety."

The hypersensitive response (HR) (*1, 2*) is a common mechanism by which plants defend themselves. In the HR response, a localized necrotic lesion forms within a small zone surrounding the site of infection, and subsequent, systemic spread of the invading pathogen beyond a few cells surrounding the necrotic zone is restricted. In addition to the local defense response, HR also induces Systemic Acquired Resistance (SAR) in other tissues of the same plant (*3*) that retards initial infection and cell-to-cell spread in subsequent infections by the same and other pathogens (*4*). Harpin$_{Ea}$ is the first bacterial HR elicitor to be discovered (*5*). It was isolated from *Erwinia amylovora*, a causal agent of fire blight in pear, apple and other rosaceous plants. Subsequently, harpin or harpin-like proteins have been isolated and characterized from many other bacterial plant pathogens (*6, 7*).

These HR elicitors constitute a previously undescribed class of proteins. The harpin family proteins share common characteristics. They are heat-stable, glycine rich, acidic (low pI), have no cysteine, and are secreted via the type III secretion pathway (*8*). The harpin$_{Ea}$ from *Erwinia amylovora* consists of 403 amino acids with a molecular weight of ca. 40 kDa. The gene encoding the harpin$_{Ea}$ protein is contained in a 1.3 kb DNA fragment located in the middle of the *hrp* gene cluster (*5*). Beyond its ability to elicit the HR in a wide range of plant species, it was demonstrated that, when applied as a topical spray, harpin protein induces plant defense and growth responses without visible HR (*9, 10, 11, 12*).

Messenger® is the first commercial product of a fundamentally new crop production technology born out of basic research conducted in the early 1990's. One objective of this research was to further elucidate plant–pathogen interactions. While it has long been understood that plants have complex mechanisms that enable them to recognize and respond to pathogen infection, scientists have sought ways to "switch on" a plant's natural defense and growth systems for the benefit of commercial agriculture. The discovery of harpin proteins and the subsequent development of Messenger now make this possible. Using topical application of Messenger, or delivery of harpin proteins through transgenics, one can harness harpin technology as a signal to activate plant systems in ways that ultimately lead to greater crop yields and quality.

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Harpin$_{Ea}$ Activates Multiple Plant Defense and Growth Pathways

Plants have evolved multiple defense pathways in responses to pathogen attack. Two of the best-characterized pathways are the salicylic acid and jasmonate/ethylene dependent pathways (*3*, *13*). Expression of the pathogenesis related protein PR1 is regulated via the salicylic acid dependent pathway and serves as a marker for activation of the SA pathway. PDF1.2 encodes a plant defensin and is dependent on the jasmonate/ethylene dependent pathway (*14*). Expression of both PR1 and PDF1.2 genes was up regulated in harpin$_{Ea}$-treated plants relative to control plants indicating that harpin$_{Ea}$ activates both the SA-dependent and jasmonate/ethylene dependent defense pathways. Induction of both PR1 and PDF1.2 was rapid. Both were detectable within six hours (data not shown) after treatment and reached maximum expression between 12 and 48 hours (Figure 1). Activation of both pathways by harpin$_{Ea}$ differed from the effect of the salicylic acid analog benzothiadiazole (BTH) that gave a strong induction of PR1 and other related SAR genes but no induction of PDF1.2 (*14*, *15*).

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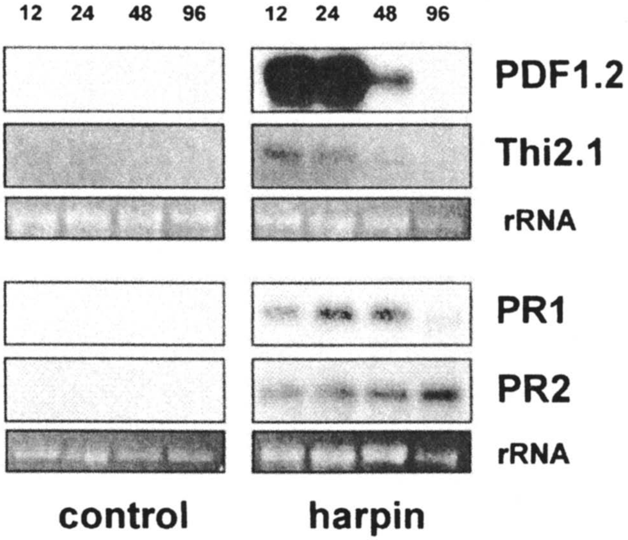

Figure 1. Defense-related gene expression in response to treatment with harpin$_{Ea}$. Four-week-old Arabidopsis plants were sprayed with a solution of 1 mg harpin$_{Ea}$ per ml or water (control) and leaves were harvested 12, 24, 48, or 96 h later. Total RNA (15 µg) was electrophoresed, blotted, and probed with a ^{32}P-labeled DNA fragment of the PDF1.2, Thi2.1, PR1 or PR2 gene. Ribosomal RNA stained with ethidium bromide served as a loading reference.

The *npr1* mutation blocks the SA-dependent pathway and prevents expression of PR1 in response to salicylic acid or pathogens (*16*). PR1 was not induced in harpin$_{Ea}$ treated *npr1* plants indicating that harpin$_{Ea}$ does act through the SA-dependent pathway. PR1 induction in *ein2* (ethylene insensitive) and *jar1* (jasmonate insensitive) mutants (*17, 18, 19*) was equal to that in wild type plants treated with harpin$_{Ea}$, which is consistent with the SA dependent pathway induction of PR1 by harpin$_{Ea}$. Induction of PDF1.2 by harpin$_{Ea}$ was not blocked by the *npr1* mutation. Induction of PDF1.2 was blocked in *ein2* plants but not in *jar1* plants. This indicates a partial dependence on the jasmonate/ethylene dependent pathway for PDF1.2 induction (Figure 2).

Resistance to bacterial infection is thought to be mediated by the SA-dependent pathway. Activation of the SA-dependent pathway by harpin$_{Ea}$ was reflected by growth inhibition of *Pseudomonas syringae* pv. tomato DC3000 in harpin$_{Ea}$-treated *Arabidopsis* plants relative to untreated controls. Resistance to Pst DC3000 was blocked in *npr1* plants but not in *ein2* or *jar1* plants confirming the role of the SA-dependent pathway in mediating at least some effects of harpin$_{Ea}$ (Figure 3).

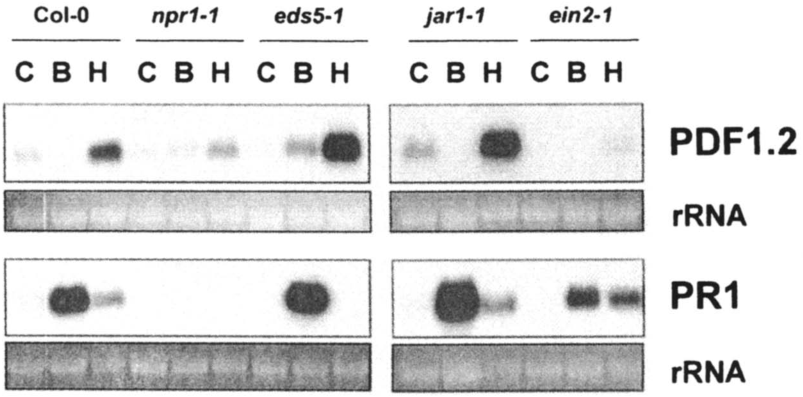

Figure 2. Effects of the npr1-1, eds5-1, jar1-1 and ein2-1 mutations on PDF1.2 and PR1 expression in response to treatment with harpin$_{Ea}$ or BTH. Four-week-old Arabidopsis ecotype Columbia (Col-0), npr1-1, eds5-1, jar1-1, and ein2-1 plants were sprayed with a solution containing 1 mg harpin$_{Ea}$ (H) per mL, 0.25 mM BTH (B), or water (C) 24 h prior to harvest. Total RNA (15 μg in the blot probed with PR1, 20 μg in the blot probed with PDF 1.2) was electrophoresed, blotted, and probed with a ^{32}P-labeled DNA fragment of the PR1 or PDF1.2 gene. Ribosomal RNA stained with ethidium bromide served as a loading reference. Results were replicated in 7 experiments with harpin$_{Ea}$ and 2 with BTH.

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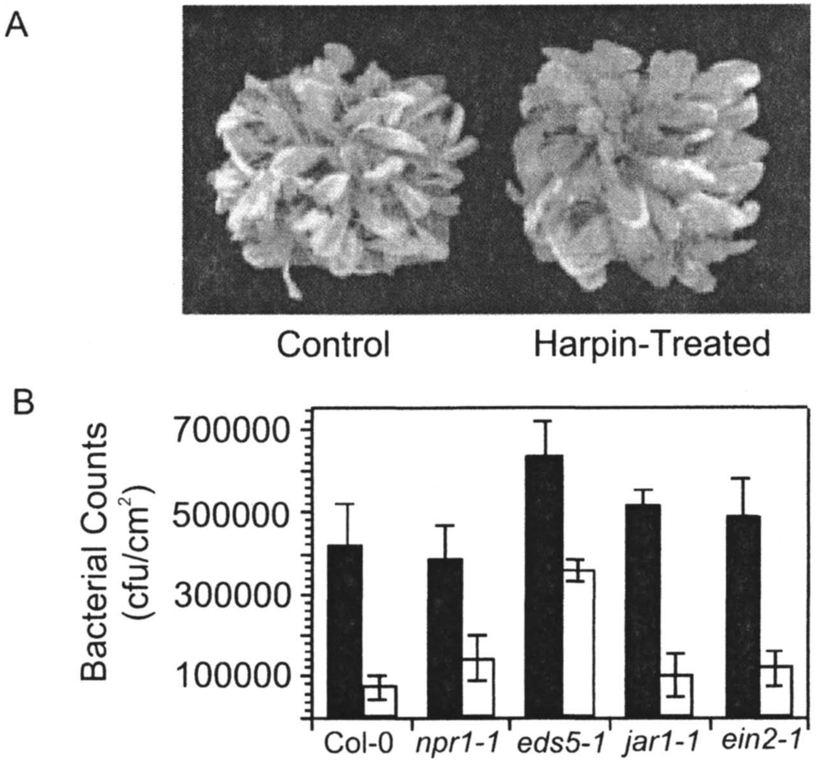

Figure 3. Growth of Pseudomonas syringae *pv.* tomato *in harpin$_{Ea}$-treated wild type and mutant* Arabidopsis. *Four-week-old* Arabidopsis *ecotype Columbia (Col-0) plants (A), or (B) Col-0,* npr1-1, eds5-1, jar1-1 *and* ein2-1 *plants were sprayed with a solution of 1 mg harpin$_{Ea}$ per mL or water (control). Twenty-four hour later, plants were inoculated by dipping them in a suspension of 10^8 cfu/mL of* Pseudomonas syringae *pv.* tomato. *(A). Plants were photographed 5 days after inoculation. (B). Bacterial counts were determined 3 days after inoculation. Open bars correspond to harpin$_{Ea}$ treatment, solid bars to water treatment. The height of the bar represents the mean value obtained for 3 replicates of 6 leaves from each treatment; error bars indicate standard error of the mean. Cfu, colony forming unit.*

Harpin$_{Ea}$ also has a profound effect on plant growth including improved root development, and increased yield and crop quality. Recently researchers have demonstrated the unique growth aspects of harpin$_{Ea}$ in a variety of experiments. Harpin$_{Ea}$ treated plants showed substantial increases in net photosynthesis (Figure 4) and nutrient uptake.

Global changes in gene expression were assessed in a preliminary microarray experiment in an attempt to obtain insight into the multiple effects that result from topical application of harpin$_{Ea}$. A substantial number of genes are significantly up or down regulated by harpin$_{Ea}$. Many genes fell into categories related to specific functions including defense genes encoding various pathogenesis related proteins (PR proteins), the phenylalanie amonia lysate (PAL) pathway, oxidative burst, jasmonate synthesis pathway, protein transport, polyamine synthesis, cell wall development, cell elongation and photosynthesis. A number of the induced genes were of unknown function, including genes with with and without plant homology.

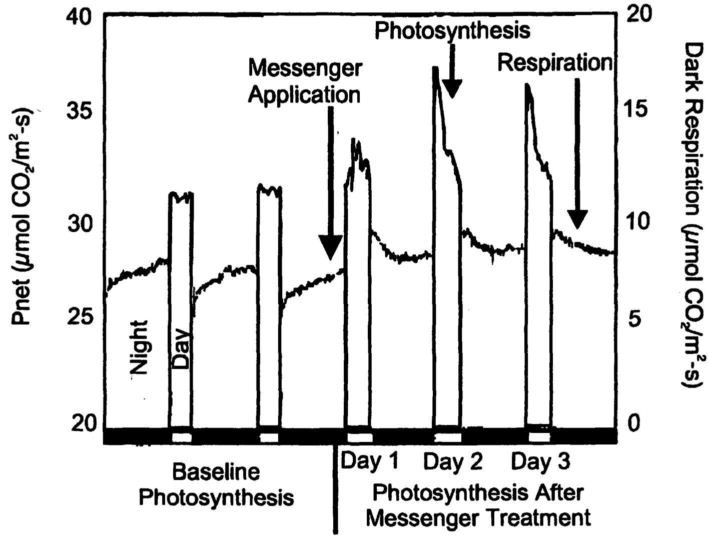

Figure 4. Messenger treatment increases daytime photosynthesis and nighttime respiration in wheat plants. Wheat plants were grown through anthesis phase in a closed system growth chamber (NASA Ames Center) permitting continuous measurement of photosynthetic activity. Photosynthetic rates were monitored both before and after treatment with Messenger. Baseline measurements were made to establish the pre-treatment level of photosynthetic activity. Leaves were sprayed with Messenger solution (20 ppm harpin protein) 30 minutes prior to the beginning of the daily photoperiod. Day 1, 2, and 3 represent the days following Messenger application.

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HrBP1, a Harpin$_{Ea}$ Binding Protein, may Mediate Plant Defense and Possibly Plant Development and Growth

By using the yeast two hybrid screening system, a harpin$_{Ea}$ binding protein gene was isolated from *Arabidopsis thaliana* and many other plants. The gene encoding HrBP1 is on chromosome 3. The genomic sequence consists of four exons and three introns; exon 4 includes a 130 bp non-translated 3' region. The open reading frame encodes a polypeptide (named HrBP1p) of 284 amino acids. The predicted molecular weight of HrBP1p is 30.45 kDa and the pI is 5.72. The protein is predicted to be noncytoplasmic and possibly located outside of plant cells.

The genes encoding HrBP1-like proteins are expressed in a wide range of plant species (Figure 5). Homologs of HrBP1 were cloned from rice, wheat, barely, corn, cotton, tobacco, tomato, potato, soybean, apple, grape and grapefruit. The deduced HrBP1 protein sequences are highly conserved. We believe that at least certain harpin-mediated signal transduction is initiated through the interaction between harpin and HrBP1. The biological function of HrPB1 in plant defense and growth is currently under investigation.

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Messenger: The First Product Developed from Harpin$_{Ea}$ Protein

Messenger is the first product developed from harpin$_{Ea}$ protein. It is a wettable fine granule comprised of 3% harpin$_{Ea}$ protein formulated with food grade ingredients (Figure 6). The product is manufactured through an environmentally safe, water-based fermentation process that has no harmful chemical intermediates or additives, followed by partial protein purification, then drying and agglomeration with carrier. Harpin protein has no direct anti-microbial activity; therefore, disease suppression by application of Messenger does not result from the direct killing of a pathogen but rather from the activation of a plant's natural resistance and growth mechanisms.

Messenger can be applied as a foliar spray with conventional application equipment. It can also be applied as a seed treatment or as a root drench in a green house. Repeat applications are required for dicots such as citrus, grape and fresh vegetables. However, certain monocots, such as wheat and rice, need only one or two applications per season. Full activation of plant defense and growth systems may take 5-7 days after application of Messenger, but the initiation of the response occurs rapidly. Once the systems are initiated, the continued physical presence of Messenger on plants is not required.

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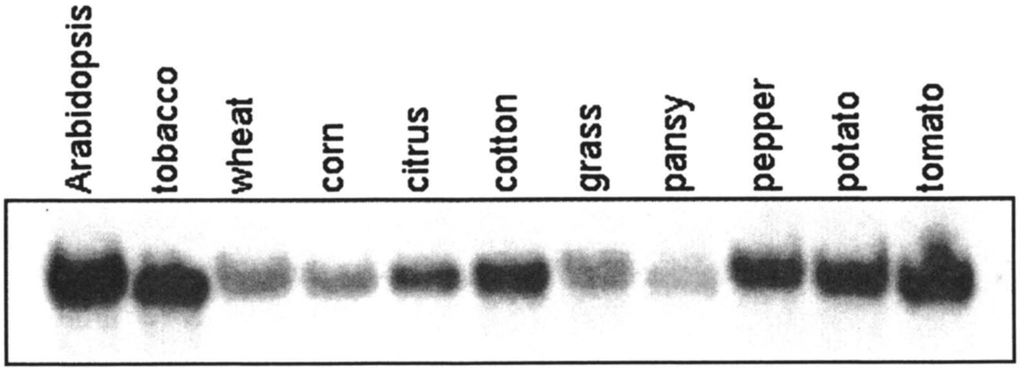
Arabidopsis
tobacco
wheat
corn
citrus
cotton
grass
pansy
pepper
potato
tomato

Figure 5. An HrBP1*-like transcript exists in all plants tested. Total RNA was extracted from leaf tissue of various plants and separated on agarose gels and blotted. Northern hybridization was performed using a RNA probe complementary to bases 651 to 855 of the* At HrBP1 *cDNA-coding region.*

Messenger Enhances Crop Growth, Yield and Quality

Messenger significantly enhances plant growth by activating plant growth systems including increased nutrient uptake and photosynthesis. World-wide field trials have shown that application of Messenger results in growth enhancement as evidenced by one or more of the following: increased seedling stand, better root development, increased plant biomass, increased fruit quality or increased yield (Figure 7). Messenger-treated citrus and tomato plants not only display enhanced growth and development, but also larger fruits with extended shelf life. Harpin protein applied pre- or post-harvest to apples significantly reduced the progress of post-harvest disease (Blue Mold, *Penicillium expansum*) over time compared to untreated controls (*20, 21*). Yield increases generally range from 10-20% in Messenger-treated crops including citrus, tomato, cucumber, pepper, strawberry and table grape. For example, strawberries treated with 4.5 oz/A Messenger at 14-21 day intervals in eight independent field trials yielded 12 percent more fruit on average than untreated plants (Figure 8). Early applications of Messenger improved strawberry root mass and stand establishment (Figure 9) and increased the number of early season blooms by 14 percent.

Messenger's ability to improve plant health and quality is also being recognized by users outside of commercial agricultural production. Consumer interest has been strong after the introduction of a Messenger "Home & Garden" product in early 2003. The American Rose Society has evaluated the product and given Messenger its official endorsement. University extension research on greenhouse-grown ornamentals concluded that Messenger treated plants had better growth than untreated controls and that most of the treated plants were more marketable (*22*). Recently, Eden Bioscienc introduced Messenger product labeling and packaging targeted for the commercial turf, ornamentals, and greenhouse market.

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Messenger is Virtually Non-toxic and Environmentally Safe

In the U.S., Messenger is classified and regulated by the EPA as a biochemical pesticide, a category of biopesticide products characterized by their natural occurrence and "non-toxic" mode of action. Based on independent toxicology studies, Messenger is classified by the EPA as practically nontoxic to humans and other mammals, birds, honeybees, fish, plants and aquatic species (Table 1). The product exhibits no demonstrable skin or eye irritation, hypersensitivity or allergic reaction, and is designated a Toxicity Category IV product for all routes of potential human exposure – the lowest hazard category possible for a pesticide. No adverse environmental or health effects have been observed after four years of commercial use in agriculture.

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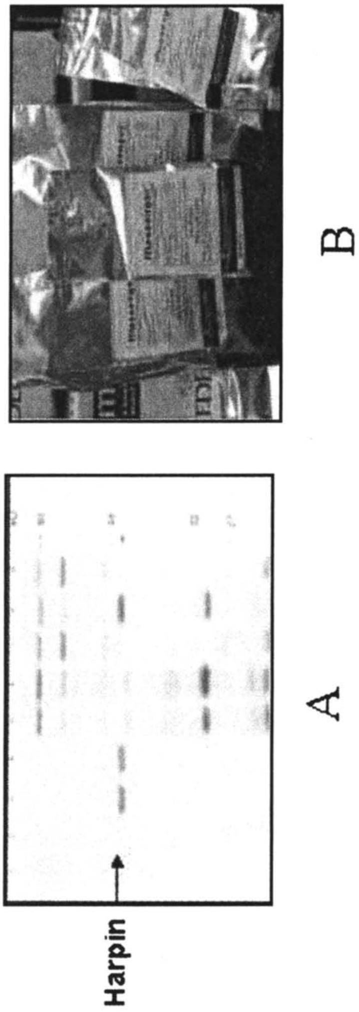

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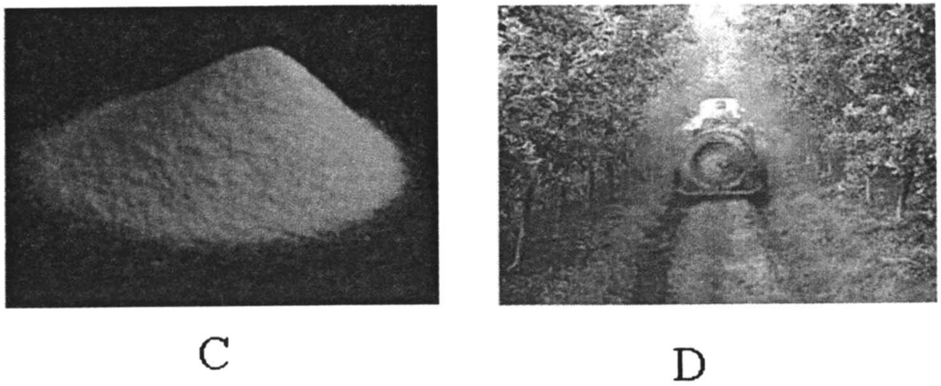

Figure 6. Messenger containing active ingredient harpin protein is a new tool for integrated crop management. A Purified harpin protein shown in SDS gel; B. Commercial package of Messenger product; C. Wettable fine granule Messenger; D. Application of Messenger to plants with a conventional sprayer.

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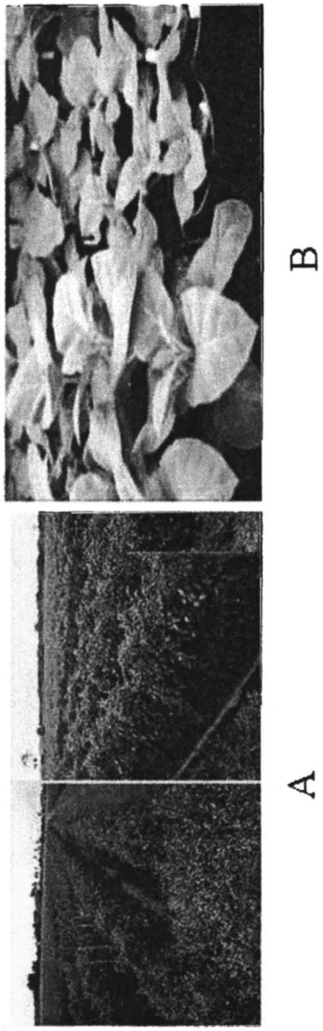

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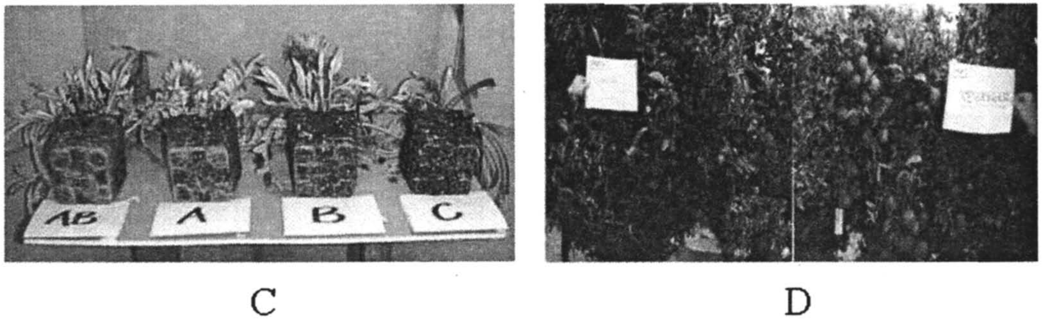

Figure 7. Messenger enhances growth and increases production in a wide range of crops. A. Large field trial of tomato showing healthier and more vigorous growth from the Messenger treated tomato (right half panel); B. Tobacco following seed treatment and one foliar application of Messenger in green house. Left half panel is Messenger treated plants and right half panel is untreated control; C. Plants (Daybreak) showing better and stronger root development resulting from Messenger treatment (AB, A and B; C is a control plant); D. Citrus trees showing alternance break in the second year fruit bearing from Messenger application. Messenger treated tree is in the right half panel and grower standard is on the left.

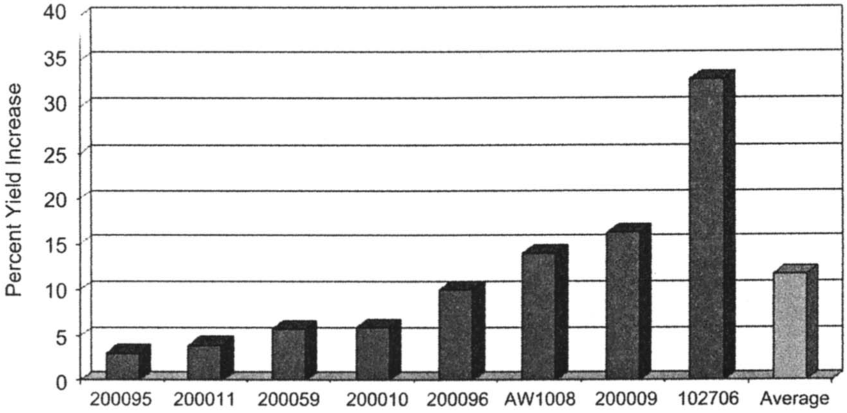

Figure 8. Strawberry yield increases in different trials following foliar applications (4.5 oz/A) of Messenger at 14-21 d intervals near the beginning of production.

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In contrast to the strong element of health and environmental risk associated with the use of synthetic chemical pesticides, there is no risk associated with the use of Messenger. While much personal protective equipment (respirators, rubber gloves, boots and complete suits of protective outerwear) is required when applying chemical pesticides, no protective equipment is necessary for users who apply Messenger. Workers may re-enter Messenger-treated fields after only 4 hours – the minimum re-entry interval. Furthermore, harpin protein is exempt from residue tolerances on all crops. In summarizing its safety assessment for harpin protein, the EPA concluded that: "Because of the lack of demonstrated adverse health effects, low rates of application, and rapid degradation in the field, no residues are expected on treated crops and attendant dietary risks are expected to be minimal to non-existent. Because of the lack of demonstrable toxicity, no adverse effects are expected to applicators, handlers and other workers" (*23*).

The EPA officially recognized the potential benefits of Messenger with its Presidential Green Chemistry Award in 2001: "Messenger: A Green Chemistry Revolution in Plant Production and Food Safety." In addition to the U.S., Messenger is currently approved for use in 26 foreign countries, including Spain, Germany, China, and Mexico. In 2004, EPA granted an exemption from tolerance for all harpin proteins that meet specific safety and characterization criteria. The Agency also approved large-scale field trials for a next generation product of harpin technology, containing one percent harpinαβ protein. Commercialization of this product is expected to begin in 2005-06 under the tradename ProAct.

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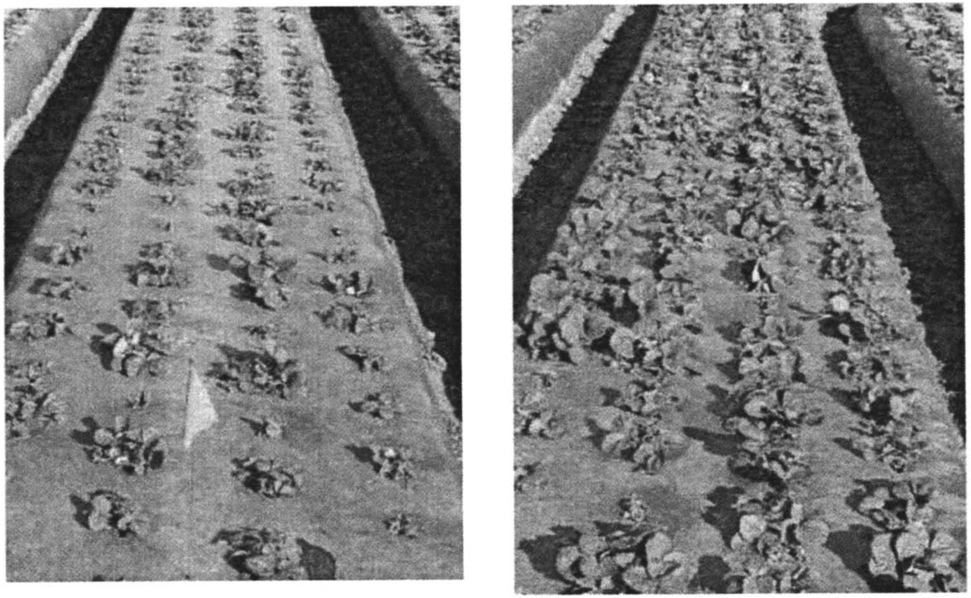

Figure 9. Messenger Assists in Stand Establishment. In trials conducted in California and Florida, early applications of Messenger improved root mass and stand establishment, bringing the crop into full production sooner.

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Table 1. Summary of Mammalian and Ecological Effects for Messenger

Toxicity Test	*Toxicity Endpoint*	*EPA Toxicity Classification*
Acute Oral (Rat)	LD50 > 5,000 mg/kg bw	IV
Acute Dermal (Rat)	LD50 > 6,000 mg/kg bw (mild dermal irritant)	IV
Acute Inhalation (Rat)	LC50 > 2.16 mg/L	IV
Skin Irritation (Rabbit)	Non-irritating	IV
Eye Irritation (Rabbit)	Non-irritating	IV
Avian Acute Oral (Bobwhite Quail)	LD50 > 4,000 mg/kg bw; NOAEC = 4,000 mg/kg bw	Practically Non-toxic (PNT)
Avian Dietary (Bobwhite)	LC50 > 100,000 mg/kg bw	Practically Non-toxic (PNT)
Fish Acute (Rainbow trout)	LC50 > 3,270 mg/L; NOAEC = 378 mg/L	Practically Non-toxic (PNT)
Aquatic Invertebrate Acute (Daphnia magna)	EC50 = 1,173 mg/L; NOAEC = 325 mg/L	Practically Non-toxic (PNT)
Acute Contact (Honeybee)	LD50 > 39 µg harpin/bee NOAEC = 39 µg/bee	Practically Non-toxic (PNT)
Seedling Emergence	No Phytotoxicity	Not Applicable

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Chapter 15

Soil Fate and Non-Target Impact of Bt Proteins in Microbial Sprays and Transgenic Bt Crops

Graham Head

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Laboratory and field testing of the soil fate and non-target impact of Bt Cry proteins in the form of microbial sprays and plant tissues from transgenic Bt crops indicate that these proteins break down relatively rapidly in soil, do not bioaccumulate, and have few or no detectable effects on non-target organisms. In contrast, other components of microbial sprays such as Bt spores can persist for several years and may bioaccumulate. These components of sprays, and other formulation ingredients, can have limited non-target impacts. However, both microbial Bt sprays and transgenic crops expressing Bt proteins have minimal impact on non-target species relative to commonly used conventional insecticides. Thus microbial Bt sprays and Bt crops can form the basis for sustainable IPM programs.

The common soil bacterium *Bacillus thuringiensis* (Bt) has been shown to produce a wide variety of insecticidal proteins. Different insecticidal proteins are produced by different strains of Bt and any given strain may produce different proteins at different points in their life cycle. The group of Bt proteins that have received the most attention are the so-called crystalline (Cry) δ-endotoxins. Over 30 classes of Bt Cry proteins have been identified and classified based on sequence identity and spectrum of activity. For example, Cry1 proteins are active against various Lepidoptera, Cry2 proteins are active against a set of Lepidoptera and Diptera, and Cry3 proteins are active against

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

certain Coleoptera (*1,2*). A number of Cry1, Cry2, and Cry3 proteins have been used as environmentally benign insecticides in agriculture and forestry for over 30 years to control several key pest Lepidoptera and Coleoptera. However, the scale of use has been relatively limited because they generally only provide partial control of pest populations. The Bt Cry proteins in microbial formulations are relatively slow acting and tend to break down rapidly when exposed to ultraviolet light and high temperatures.

Within the last 10 years, technologies have been developed that enable Bt proteins to be used more efficaciously in agriculture. The genes coding for the Cry1Ab, Cry1Ac, Cry1F, Cry2Ab2, Cry3Aa and Cry3Bb1 proteins, among others, have been synthesized and successfully inserted into crop plants through recombinant biotechnology, producing transgenic crops that express the relevant proteins (so-called Bt crops). Expression of these proteins in crop plants has been optimized so that the Bt Cry proteins generally are present at high levels in all of the plant parts that are vulnerable to target pest consumption. These high levels of expression, and the stability in expression over time that results from the proteins being protected from ultraviolet degradation, mean that Bt crops can be extremely effective in the level of pest control they achieve, thereby overcoming the difficulties associated with microbial sprays. At the same time, only organisms that feed on plant tissues will be exposed to the Bt proteins in Bt crops, meaning that potential non-target organism effects are minimized.

Thus far, the commercial applications of Bt crops include expressing Cry1Ab and Cry1F in corn for lepidopteran pest control, Cry3Bb1 in corn for control of corn rootworm species (*Diabrotica spp.*), Cry1Ac and Cry2Ab2 in cotton for lepidopteran pest control, and Cry3Aa in potato for control of the Colorado potato beetle, *Leptinotarsa decemlineata*. Bt corn varieties are now grown on over 52 million acres in countries that include the U.S., Canada, Spain, Argentina, South Africa and the Philippines. Bt cotton varieties are grown on over 37 million acres in countries that include the U.S., Mexico, Argentina, Colombia, Australia, India and China (*3*).

The widespread use of Bt row crops in agriculture and of microbial Bt sprays in organic agriculture and forestry makes it critically important that the impact of Bt proteins from either of these sources on agroecosystems is thoroughly understood. This paper focuses on potential impacts of Bt proteins from these different sources on soil organisms, reviewing available literature from laboratory tests, field experiments and longer-term monitoring. For there to be ecological impacts on non-target organisms from the use of Bt proteins, both exposure and hazard must be present. Therefore, a simple ecological risk assessment approach is used that separately considers the potential exposure of soil organisms to Bt proteins from various sources and the potential hazards posed by Bt proteins to soil organisms. Finally, the non-target impacts of Bt proteins are contrasted with the demonstrated impacts of alternative pest control practices.

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Potential Exposure of Soil Organisms to Bt Proteins

Movement of Bt Proteins into the Soil

Potential routes of exposure to Bt Cry proteins will be rather different for Bt microbial sprays compared with Bt crops (*4*). For microbial sprays, exposure of soil organisms will result from some portion of the spray application falling directly upon the soil surface or being washed into the soil by rain or irrigation. Degradation of the Bt proteins in microbial sprays tends to be rapid, though encapsulation of the Cry protein can slow degradation by reducing exposure to ultraviolet radiation and other environmental factors (*5*). Overall, this means that movement of Bt proteins from microbial sprays into the soil and exposure of soil organisms will occur primarily at or shortly after the time of spray application.

In contrast, Cry proteins from Bt crops can enter the soil in three ways. First, tillage of plant material into the soil at the end of the season can introduce Bt proteins into the soil. However, Bt protein levels in the tissues of Bt crops tend to decline as the plant senesces (*6*). Thus, the amount of Bt protein being introduced into the soil by tillage of Bt crops at the end of the season will be relatively small. Second, plant material may fall sporadically from live plants during the season, including leaves dropping throughout the year and pollen deposition during anthesis. The amount of plant material involved will be small compared to end of season tillage. Furthermore, expression of Bt proteins tends to be relatively low in the pollen of Bt crops (*7*). Third, samples of soil from the rhizosphere of Bt corn seedlings under various conditions suggest that Bt protein may be exuded from the roots into the rhizosphere (*8*), but sluffage of root tissue containing Bt protein may be a confounding factor for the results obtained.

Collectively, the available data indicate that Bt proteins from either microbial sprays or Bt crops may enter the soil in various ways, though in small amounts. For example, conservative calculations indicate that end of season tillage of a Bt cotton field results in <2 g of Cry1Ac protein per acre entering the soil. If all of this material were introduced into just the top three inches of soil, this would result in approximately 0.1 ppm of Cry1Ac in this soil layer (*9*). For comparison, hazard testing with Cry1Ac against non-target organisms has been performed at concentrations ranging from 20-200 ppm (see below) and no adverse effects have been observed (*7*).

Persistence of Bt Proteins in the Soil

Given that Bt proteins can enter the soil via a number of routes, we need to know whether those proteins are capable of persisting and even accumulating in

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soil. The amount of data addressing this issue is substantial (*10-18*). Relevant studies have been carried out by various public sector laboratories, as well as by the private sector as part of the data requirements of the U.S. Environmental Protection Agency (EPA) for registration of Bt crops. Table I shows the estimated half-lives (in days) of particular Bt Cry proteins in soil. The estimates were based on the amunt of Bt protein originally present following the incorporation into soil of a microbial preparation of Bt var. *kurstaki*, an individual purified Bt protein, or the Bt protein in Bt crop plant tissue. These studies indicate that the half-lives of Bt proteins are typically short, regardless of the form in which they are introduced; Bt proteins contained within microbial preparations, various isolated Bt proteins (that could have come from microbial sprays or from Bt crop plants), and Bt proteins in the plant tissues of a Bt crop had half-lives of <20 days in all but two cases, with no indication that substantial amounts of protein remained undegraded in the soil. The rates of degradation were comparable for Bt proteins expressed in plant tissues and microbial Bt proteins.

Experiments with sterilized soil have demonstrated that Bt protein degradation is driven by microbial activity (*14*). Thus much of the variability seen within and among studies may reflect differences in microbial activity caused by the use of different soil types (*19*), maintaining samples at different temperatures (*16,20*), or preparing the samples in different ways (for example, placing plant material in litter bags could slow microbial breakdown; see Table I and ref. *20*). In any case, the breakdown of Bt proteins appears to be rapid under any conditions of significant microbial activity.

Table I. Half-lives (days) of Bt Proteins from Various Sources

Class	*Purified Protein*	*Transgenic Plant*	*Reference*
Bt isolates	2.7-7[a]		10,11
Cry1Ab (corn)	8.3	1.6	15,18
Cry1Ab (cotton)	17	4	13,14
Cry1Ac (cotton)	9-20	7-62	12-14,18
Cry1F (corn)	<1		17
Cry2Aa (cotton)		15.5-31.7	16
Cry2Ab2 (cotton)	1.1-3.5		18
Cry3Bb1 (corn)	2.4-2.8		18

[a] Bt isolates contained spores and parasporal crystals.

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Accumulation of Bt Proteins in the Soil

When Bt proteins are introduced into soil, some small portion of the introduced protein binds rapidly to clay particles and humic acids within the soil, apparently becoming inaccessible to microbial degradation in the process (*21*). Thus, even if Bt proteins are degraded rapidly in soil, it is possible that the small amounts of Bt protein that bind to soil particles may lead to accumulation of Bt protein in soil over time with repeated use. However, studies of cotton fields in Mississippi and Alabama in which Bt cotton expressing Cry1Ac had been grown for 3 to 6 consecutive years failed to detect any Bt Cry protein by either immunological or insect bioassay methods, despite regular tillage of plant material into the soil over the previous years (*9*). Similarly, studies of the soil in cornfields in which Bt corn expressing Cry1Ab had been grown for 4 years detected trace amounts of the Cry1Ab protein (*22*). The Bt protein was primarily in the form of undecomposed plant residues, particularly early in the growing season. Overall, the results indicated that almost all of the Bt protein from the Bt corn was degraded within several weeks with no evidence of build up over time. Any Bt protein bound to soil particles was biologically unavailable or inactive. Thus, there is no indication that Bt proteins bioaccumulate in soil.

Persistence of Other Components of Microbial Bt Sprays in the Soil

Microbial Bt sprays typically contain components other than Bt Cry proteins, including Bt cells and spores as well as formulation ingredients unrelated to Bt. These components may be more resistant to degradation than the Cry proteins, and thus may persist and even accumulate in soil. In particular, intact Bt cells and spores may survive, establish and reproduce in suitable soils. Studies of soil from citrus orchards in China (*23*), forests in Canada (*24*), and cabbage fields in Denmark (*25*), all of which had been intensively sprayed with microbial Bt formulations, demonstrate that Bt cells can persist for several years or much longer after applications have ceased. In these cases, the persistence of Bt cells is a dynamic process involving germination, cell division, and sporulation in specific microhabitats. Comparisons of Bt spore populations and Bt protein levels in soil over time clearly indicate that the Cry proteins are degraded far more rapidly than the spores (*10*).

Overall Exposure of Non-target Soil Organisms to Bt Proteins

Given the relatively rapid degradation of Bt proteins in soil regardless of their source and the fact that they do not accumulate, exposure of soil organisms to Bt proteins generally will be minimal. The only possible exceptions would be

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when highly susceptible non-target organisms are present at times of maximum movement of Bt proteins into soil. This would be at the time of microbial spray application or possibly on the rare occasion when actively growing Bt crop plant material with a high concentration of Bt protein (unlike pollen or senescent material) is tilled into the soil. Microbial formulations with live Bt cells and other microbial material may pose greater exposure risks to soil organisms because they are capable of persisting for long periods in the soil at significant levels.

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Potential Hazard of Bt Proteins to Soil Organisms

The spectrum of activity of each microbial Bt formulation and each Bt protein expressed in a transgenic Bt crop has been determined individually as part of EPA's data requirements for registration of such products. In addition, each microbial Bt formulation and each Bt protein expressed in a Bt crop has been tested against representative non-target, beneficial insects [including honey bee (*Apis mellifera)*, ladybird beetle (*Hippodamia convergens*), green lacewing (*Chrysopa carnea*) and a parasitic wasp (*Brachymeria intermedia* or *Nasonia vitripennis*)] and two key non-target soil invertebrates [earthworm (*Eisenia fetida*) and Collembola (*Folsomia candida*)]. The results of these tests indicate that none of the Cry proteins engineered into Bt crops or commonly used in microbial Bt formulations has activity against these taxa (*7,26*).

As noted earlier, only Cry1, Cry2 and Cry3 proteins are currently used for pest control in agriculture; the Cry1 proteins have only been found to have lepidopteran activity, the Cry2 proteins have lepidopteran and dipteran activity, and the Cry3 proteins only have limited coleopteran activity (effects have only been observed with chrysomelids). Thus, even if substantial amounts of Bt protein were to persist and accumulate in soil (by mechanisms not previously observed), given what is known about the spectrum of activity of Cry1Ab, Cry1Ac, Cry1F, Cry2Ab2, Cry3Aa and Cry3Bb1, no activity is expected against the invertebrate species that are important to soil processes.

Additional published laboratory and greenhouse studies confirm the specificity of the Bt proteins present in microbial Bt sprays and transgenic Bt crops. For example, even the coleopteran predator, *Coleomegilla maculata,* was not affected by feeding on pollen from Bt corn expressing the coleopteran-active protein Cry3Bb1 (*27*). Other studies have shown that several species of Collembola are not susceptible to microbial Bt formulations (28) or a variety of Cry1, Cry2 and Cry3 proteins (29,30). Similarly, representative species of earthworms, nematodes, protozoa, bacteria, and fungi were not impacted by the Cry1Ab protein from Bt corn (*31*). In particular, no significant differences were observed in the survival and weight of earthworms (*Lumbricus terrestris*) after 40 days in soil planted with Bt or non-Bt corn or after 45 days in soil amended with biomass from Bt or non-Bt corn. Likewise, no significant differences in the colony-forming units of culturable bacteria (including actinomycetes) and fungi, and in the numbers of protozoa and nematodes, were seen between the rhizosphere soil of Bt and non-Bt corn or between soil amended with biomass from Bt and non-Bt corn. Other studies of the impact of purified Bt proteins or Bt plant tissue expressing either Cry1Ac or Cry3Aa on soil microbial communities found transient effects apparently caused by the addition of plant material but no effects that could be related to the Bt proteins (*32,33*).

Thus the substantial amount of available data demonstrates that the Bt proteins in microbial sprays and Bt crops pose no significant hazard to soil non-target organisms. It is possible that certain formulation components of microbial sprays may adversely impact certain non-target taxa, but these effects also appear to be minimal at the field level (see below).

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Relative Risk Posed by Bt Proteins to Soil Organisms, and the Role of Bt Protein-Based Products in IPM

A potential risk is present where exposure to a demonstrated hazard may occur. In the context of the potential impact on soil organisms of Bt proteins in the form of microbial sprays or Bt crops, the data indicate that both the potential hazard and the potential exposure posed by Bt proteins will be minimal, and thus the risk posed by these products to non-target soil organisms also must be minimal.

Field studies that integrate hazard and exposure confirm this conclusion of minimal risk (*7,26*); no adverse effects of Bt crops on non-target soil organisms have been observed in any of these studies (for example, *34-38*). Of particular interest are observations of non-target ground-dwelling Coleoptera in plots of Bt crops that express coleopteran active Bt Cry proteins. In these cases, the taxa being observed (for example, carabids and staphylinids) are in the same order as the target pest species, though in different families. For both corn expressing the Cry3Bb1 protein and potato expressing the Cry3Aa protein, no adverse impacts were observed on these non-target predatory Coleoptera (*36,37*). This demonstrates just how specific these Bt proteins are in their effects.

Numerous field studies with microbial Bt sprays (in a few cases, the same studies that also looked at Bt crops) also have found few or no adverse effects on non-target soil organisms, including insects, other arthropods and microbes (for example, *24,37-40*). Where impacts have been observed, these have been attributed to components of the microbial Bt sprays other than the Cry proteins. For example, collembolan and earthworm species were adversely affected by the oil-based "inert" components of one microbial Bt spray formulation (*24*).

In comparison with microbial Bt sprays or Bt crops, many commonly used conventional insecticides can have dramatic adverse effects on many important non-target taxa. For example, comparisons of the effects of methyl-parathion, malathion, toxaphene, carbaryl, and a preparation containing Bt on the parasitoids *Brachymeria intermedia*, *Campoletis sonorensis*, *Chelonus blacksburni*, *Meteorus leviventris* and *Voria ruralis*, and the predators *Chrysopa carnea* and *Hippodamia convergens,* observed an average mortality rate of 27% for all species with the chemical insecticides versus less than 4% with the Bt formulation (*41*). Similar studies with five insecticides looking at their effect on various earthworm species indicated that most were moderately to highly toxic to the earthworms (*42*). Thus, replacing such broad-spectrum insecticides with microbial Bt sprays or Bt crops could benefit many non-target taxa, including various soil organisms. Field studies comparing Bt crops and microbial Bt sprays with conventional insecticide alternatives demonstrates that this is the case; studies in corn, potato and cotton have found significantly higher non-target populations in the Bt-based treatments compared with fields treated with conventional insecticides (*37,40,43*).

The consequences of the relatively higher non-target populations in Bt crop fields and those fields treated with Bt sprays can include improved secondary pest control (where predators and parasitoids are involved) and better soil quality (if decomposers are impacted). Thus, Bt-based technologies are extremely valuable components in integrated pest management (IPM) programs. In cropping systems with heavy pest pressure and consequently high insecticide use, reducing broad-spectrum insecticide use is a critical part of successful IPM. The introduction of Bt cotton is an example of this process in action. By dramatically reducing the need for (and use of) insecticidal control of lepidopteran pests in cotton, the adoption of Bt cotton in the U.S., Australia and China has led to substantial increases in non-target populations in cotton systems, and reduced human exposure to insecticides (3,43-45). For these reasons, Bt cotton has been described as introducing a new age in IPM for cotton in parts of China and the U.S.

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Chapter 16

Photolysis of Two Pesticides Used by Organic Farmers: Sabadilla and Ryania

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Solar irradiation of the major components of the "organic" pesticides sabadilla and ryania was studied in aqueous solution. One of the sabadilla components, veratridine, degraded slowly when exposed to sunlight. Another sabadilla component, cevadine, was stable. The major components of ryania (ryanodine and dehydroryanodine) also decomposed slowly in sunlight. The major products resulted from photohydrolysis.

Introduction

Under current United States Department of Agriculture National Organic Program regulations organic farmers may use some insecticides under certain conditions. These insecticides include pyrethrum, rotenone, sabadilla and ryania. Sabadilla is a broad-spectrum insecticide made by grinding seeds of *Schoenocaulon officinale* A. Gray, a member of the lily family which grows mainly in the Andes Mountains in Mexico, Guatemala and Venezuela. It is a mixture of alkaloids whose structures are shown in Figure 1. Ryania is the dried powder of roots, leaves and stems of *Ryania speciosa* which grows in the northern part of South America and the Amazon Basin. It consists mainly of two components, ryanodine and dehydroryanodine, whose structures are shown in Figure 2. There are no EPA tolerances for either sabadilla or ryania so they may be used in any amounts that the grower needs to prevent insects from

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

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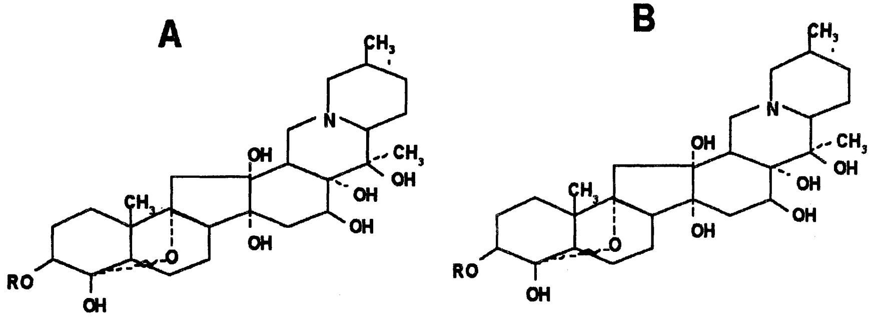


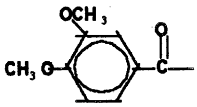
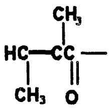
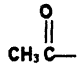
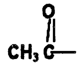
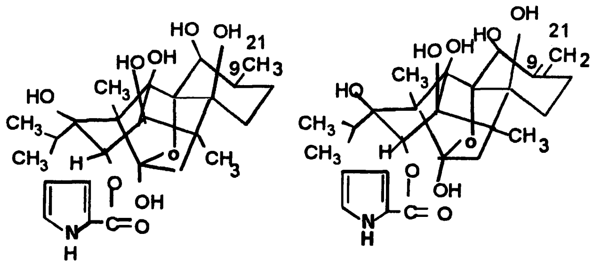
		R	MW
1 veratridine	**A**		673
2 cevadine	**A**		591
3 cevine	**A**	H	509
4 cevacine	**A**	 $CH_3C(=O)-$	551
5 sabadine	**B**	 $CH_3C(=O)-$	537

Figure 1. Structures and molecular weights (MW) of sabadilla components

destroying his/her crop. Even if there were tolerances, there would be no way to enforce them as there is no approved multiresidue procedure to determine these pesticides in food. We previously reported the development of a sensitive multiresidue procedure for pyrethrum, rotenone, sabadilla and ryania in food based on high-performance liquid chromatography (HPLC) and atmospheric pressure chemical ionization mass spectrometry (APCI/MS) (*1*).

In addition to the paucity of approved analytical methods for these pesticides, there is very little information on their chronic toxicity, their endocrine disrupter properties, and their possible enhanced effects on children.



ryanodine
(MW=493)

dehydroryanodine
(MW=491)

Figure 2. Structures and molecular weights of major components of ryania.

Nor is there much information available on the environmental fate of these materials. This report will provide preliminary results on the effect of exposure of sabadilla and ryania to sunlight in aqueous solution.

Experimental

Chemicals

Ryania (a mixture consisting of 53% dehydroryanodine and 47% ryanodine), veratrine (a mixture consisting of 59% cevadine, 38% veratridine and 3% other alkaloids), veratridine, veratric acid (3,4-dimethoxybenzoic acid) and pyrrole-2-carboxylic acid were purchased from Sigma Chemical, St. Louis, MO.

Instrumentation

HPLC and APCI/MS instrumentation and conditions have been published earlier (1). Gas Chromatography/Mass Spectrometry (GC/MS) instrumentation consisted of a Varian Model 3400 Gas Chromatograph (Varian Associates, Sunnyvale, CA) interfaced to a Finnigan ITS Magnum Ion Trap Detector (Finnigan MAT, San Jose, CA). A 30 m x 0.25 mm id DB-1 fused-silica capillary column (0.25 μm film thickness) was used. The GC was temperature-programmed from 60°-210°C at 7.5°C/min and from 210°-260°C at 5°C/min.

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Helium carrier gas velocity was 1 mL/min. Filament emission current and electron multiplier voltage were 21 μA and 1700 V, respectively.

A Waters 600 Multisolvent Delivery System connected to an Applied Biosystems 1000S Diode Array Detector was used to determine the UV spectra of veratridine, cevadine, ryanodine and dehydroryanodine.

A Rayonet Merry-go-round Photchemical Reactor (Southern New England Ultraviolet Co., Hamden CT) containing 16 UV 23W lamps, emitting UV light above 300 nm was used for the photolysis of pyrrole-2-carboxylic acid.

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Solar irradiation

Aqueous solutions (75 mg/L) of veratridine and ryania were exposed to sunlight in stoppered quartz tubes (Ace Glass, Vineland, NJ) on the roof of our laboratory building between April 22 and July 12, 1998 between the hours of 10AM and 4PM. There were only 44 days of exposure during this period as samples were not exposed on days when little sunlight was expected. An aqueous veratrine solution (20 mg/L; equivalent to 7.6 mg/L veratridine and 11.8 mg/L cevadine) was exposed for 22 days between August 18-September 15, 1998 between 10AM and 4PM, again, only on sunny days. Controls during both exposure periods consisted of the same concentrations of pesticides in the same size test tubes but wrapped in aluminum foil. Samples were analyzed periodically by removing 250 μL aliquots. The aliquots were combined with 10 μL of 100 ppm aqueous caffeine solution and analyzed by HPLC/APCI/MS (*1*).

Determination of the Major Photolytic Products of Veratridine and Ryania Solution

A Supelco Envi-18 cartridge (6mL) was conditioned with 6 mL of methanol and then 6 mL of water. The irradiated veratridine solution (after acidification to pH=3) was loaded onto the cartridge and passed through at a flow rate of 1-2 mL/min. The cartridge was dried under vacuum for five minutes, and then 3 mL methanol eluate was collected. The eluate was then dried under a nitrogen stream and treated with diazomethane (generated from 1-methyl-3-nitro-1-nitrosoguanidine and sodium hydroxide using the method of Quin and Hobbs [*2*]). NOTE: DIAZOMETHANE IS TOXIC, MUTAGENIC AND CARCINOGENIC AND SHOULD BE GENERATED ONLY IN A WELL-FUNCTIONING HOOD. Finally, the reaction mixture was dried under a nitrogen stream to remove the excess diazomethane. Ten μL of 40 ppm internal standard mixture solution (1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}) was added, and the volume was adjusted to 250 μL with acetone. The acetone solution was analyzed by GC/MS. Veratric acid aqueous solution (75 ppm) and pyrrole 2-carboxylic acid aqueous solution (75 ppm) were also derivatized by following the above procedure.

Alkaline hydrolysis of veratridine and ryania

Five mL of veratridine aqueous solution (75 ppm) was adjusted to pH 11 with NH_4OH (20-22% w/w), and stored at room temperature for one day (*3*). The solution was extracted with three 10-mL portions of methylene chloride. The extracts were evaporated to 1 mL under reduced pressure on a flash evaporator (Buchler Instruments, Fort Lee, NJ), and then dried in a gentle stream of nitrogen. The hydrolyzed product was then dissolved into 250 μL of water, and 20 μL was injected into HPLC/APCI/MS.

Five mL of ryania aqueous solution (75 ppm) was adjusted to pH 12 with 2N NaOH and then was heated at 110^0C for three hours. The hydrolysis solution was neutralized with acetic acid, and 20 μL was directly injected into HPLC/APCI/MS.

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Results and Discussion

Solar Degradation of Veratridine and Cevadine

It took about 44 days for an aqueous solution (initial concentration: 75 ppm) of veratridine to photodegrade to 48% of its initial concentration. There was only very limited degradation in the control sample. We were unable to obtain commercial samples of any of the other sabadilla components (Figure 1) but we were able to obtain veratrine, which is a mixture of cevadine (59%) and veratridine (38%). Exposure of a 20 ppm aqueous solution of veratrine (11.8 ppm cevadine and 7.6 ppm veratridine) to sunlight for 22 days resulted in a loss of 50 % of the veratridine and only 10 % of the cevadine. Analysis of the controls indicated 98% and 94% of the veratradine and cevadine were present at the end of the experiment, respectively. Given the imperfect quantification of our methods, it is reasonable to conclude that very little, if any, degradation of veratridine and cevadine occurred during storage or at the elevated temperatures on our roof. It is also reasonable to conclude that while veratridine undergoes photolysis in sunlight, cevadine does not. The UV absorption spectrum of veratridine had peaks at 222, 264 and 294 nm, while the UV absorption spectrum for cevadine showed a peak at 225 nm. Since the earth's ozone layer absorbs all wavelengths below 286 nm, it is reasonable to expect that cevadine will not undergo appreciable photolysis in sunlight in aqueous solution. In the environment, however, photosensitization of cevadine by materials that absorb above 286 nm may occur.

HPLC/APC/MS determination of the extract from the veratridine irradiation sample showed two major peaks. The peak at 31.83 min was identical in retention time and mass spectrum to standard veratridine. The peak at 20.19 min had $(M+H)^+$ ion at 510, suggesting that it was cevine (Figure 1). Cevine is a hydrolysis product of veratridine so we hydrolyzed the latter chemically. The major veratridine hydrolysis product exhibited the same retention time and mass spectrum as the major photodegradation product, strongly suggesting that cevine is one of the solar degradation products of veratridine.

If veratridine undergoes photohydrolysis to cevine, it must also be converted to the acid portion of the ester. Treatment of the irradiation mixture with diazomethane resulted in the formation of a material identified by electron ionization GC/MS as methyl 3,4-dimethoxybenzoate. A material with identical GC/MS properties was obtained by treatment of commercially-obtained 3,4-dimethoxybezoic acid (veratric acid).

Thus, the two major solar photolysis products of veratridine are cevine and 3,4-dimethoxybenzoic acid (Figure 3). If sabadilla was a new insecticide, its

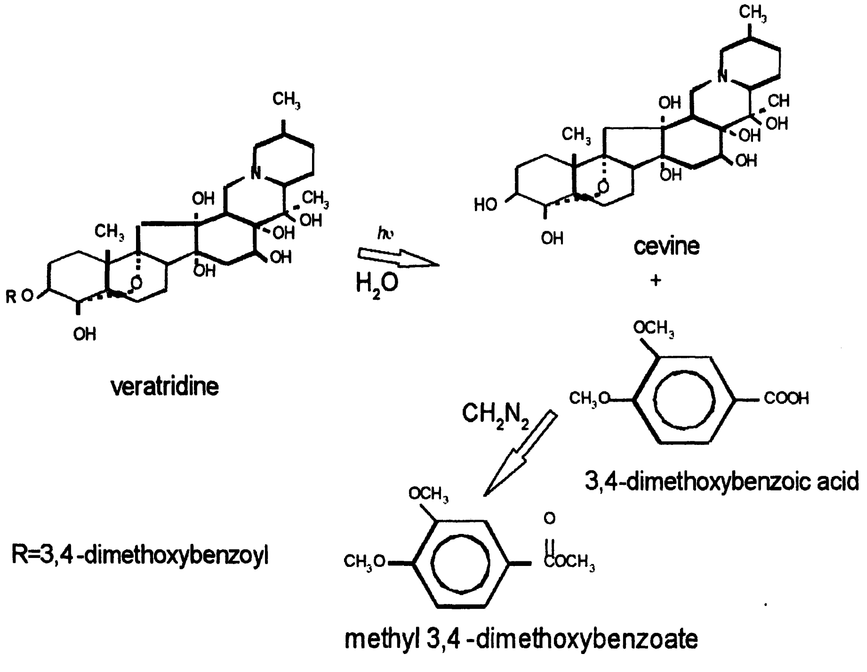
CH3
OH
CH3
OH
OH
OH
OH
RO
OH
OH
veratridine
R=3,4-dimethoxybenzoyl
hν
H2O
CH3
OH
CH
OH
CH3
OH
OH
OH
HO
OH
cevine
+
OCH3
CH3O
COOH
3,4-dimethoxybenzoic acid
CH2N2
OCH3
CH3O
O
COCH3
methyl 3,4-dimethoxybenzoate

Figure 3. Photolysis of veratridine in water and chemical methylation of 3,4-dimethoxybenzoic acid.

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manufacturer would have to provide toxicity and residue data as well as analytical procedures for its two major photoproducts.

Solar Degradation of Ryania

Solar irradiation of ryania was examined at a concentration of 75 ppm. This solution contains 35 ppm ryanodine and 39 ppm dehydroryanodine. As can be seen from Figure 2, dehydroryanodine has the same structure as ryanodine except for the absence of 2 hydrogen atoms at carbons 9 and 21. After 22 days, approximately 72% ryanodine and 68% dehydroryanodine, respectively, remained. After a 44-day exposure, 39 and 41% ryanodine and dehydroryanodine, respectively, remained. There were no significant changes for the control groups.

Identification of Major Solar Degradation Products of Ryania

Ryanodine and dehydroryanodine photoproducts were tentatively identified by HPLC/APCI/MS in the negative ion mode. The retention times of ryanodine and dehydroryanodine were 22.37 and 21.29 min, respectively, while the retention times of their corresponding photoproducts were 11.63 and 9.16 min. Because APCI in the positive ion mode did not exhibit $(M+H)^+$ ions in either ryanodine or dehydroryanodine, we operated in the negative ion mode. Ryanodine exhibited an $(M-H)^-$ ion at m/z 492 while dehydroryanodine exhibited an $(M-H)^-$ ion at m/z 490. The photoproducts exhibited $(M-H)^-$ ions at m/z 399 and 397, respectively, suggesting that they were ryanodol and dehydroryanodol plausibly resulting from the loss of the pyrrole carboxylate moiety.

Alkaline hydrolysis of ryania resulted in products having identical chromotograhic retention times as the photolysis products. Products from both reactions also exhibited identical HPLC/APCI/MS spectra in both positive and negative ion modes, further providing very strong evidence for the structure of the photoproducts (Figure 4).

Esterification of the ryania photolysate products and subsequent GC/MS analysis failed to find evidence for the presence of pyrrole-2-carboxylic acid, a material that would be expected to be formed as a result of the photohydrolysis of ryania. However, pyrrole-2-carboxylic acid was itself susceptible to rapid degradation in sunlight. After 1 hr of irradiation of pyrrole 2-carboxylic acid by UV light at wavelengths >300nm, 70% was lost, and after 4 hours none could be detected.

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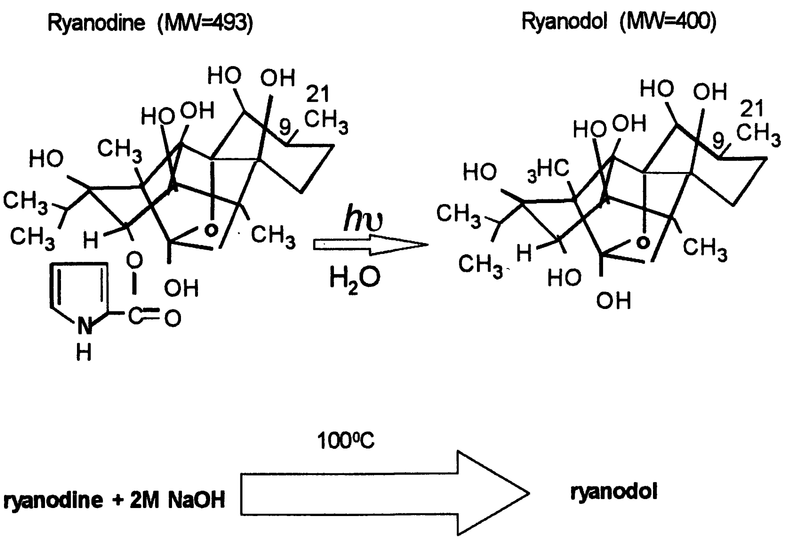
Ryanodine (MW=493)
Ryanodol (MW=400)
hυ
H2O
100°C
ryanodine + 2M NaOH
ryanodol
21
9
CH3
HO
OH
H
N
C=O
O

Figure 4. Solar (upper) and alkaline (lower) hydrolysis of ryanodine. Structure of dehydroryanodol is the same as ryanodol except for a double bond between carbon 9 and carbon 21.

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Chapter 17

Environmental Fate and Ecological Impact of Copper Hydroxide: Use of Management Practices to Reduce the Transport of Copper Hydroxide in Runoff from Vegetable Production

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Vegetable production practices combining copper-based pesticides with polyethylene mulch create conditions for highly toxic runoff emissions to surface waters. Copper hydroxide is a widely used fungicide-bactericide approved for both organic and conventional agricultural production of vegetable crops for control of diseases. Copper-based pesticides are often viewed as more "natural" than synthetic organic pesticides, but aquatic biota, such as the saltwater bivalve *Mercenaria mercenaria*, are extremely sensitive to low concentrations of copper. The use of polyethylene mulch in organic and traditional vegetable production is gaining popularity because it decreases pesticide use and warms the soil allowing for earlier crop planting, but its use also increases runoff volume and soil erosion. Two field studies were conducted to evaluate the effectiveness of management practices to reduce loads of copper in runoff from tomato production. Seasonal runoff losses of 20 to 36% of applied copper hydroxide were observed in tomato plots using plastic mulch with bare soil furrows. The addition of vegetative furrows between the raised, polyethylene-covered beds or the replacement of polyethylene mulch with vegetative residue

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ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

mulch reduced copper loads in runoff by an average of 72 and 88%, respectively, while maintaining harvest yields. Use of these alternative management practices could reduce surface water concentrations in nearby streams from the observed 22 μg/L to approximately 6 and 3 μg/L, respectively, which would be below the median lethal concentration for larval clams (*M. mercenaria* 96-h LC_{50} = 21 μg/L) and close to or below the EPA guidelines to protect aquatic life (24-h average = 5.4 μg /L for fresh water and 4.0 μg /L for salt water).

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Organic agriculture is defined as an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity. Certified organic cropland doubled during the five year period of 1992 to 1997, and in 1999, organic food retail sales reached six billion dollars (*1*). The USDA estimated ~8000 certified organic farms in 2003 were operating on 1.5 million acres of cropland and 0.75 million acres of pasture (*2*).

Copper-based materials are allowed by USDA National Organic Program (NOP) standards for the control of plant diseases in organic crop production provided they are used in a manner that minimizes copper accumulation in the soil (*3*). Copper hydroxide is a widely used fungicide-bactericide often applied prophylacticly for control of vegetable diseases. The estimated annual use of copper for fresh-market tomato production throughout the United States is 342,000 kg of active ingredient (*4*). Copper hydroxide (water solubility = 2.9 mg/L at pH 7, 25°C) readily sorbs to soil (*5-7*).

Polyethylene mulch (a thin sheet of black plastic) is a popular production practice for fresh-market vegetables and other row crops in traditional agriculture because it controls weeds and prevents soil from depositing on crops. Black polyethylene mulch also warms the soil and allows earlier planting. According to the NOP standards, mulches are allowed for use in organic crop production provided they are biodegradable or are synthetic mulches (i.e., polyethylene) that are to be removed after harvest (*3*). A 1994 survey of organic vegetable growers revealed that 21% grew fresh-market tomatoes as their main crop, and that 40 to 57% used polyethylene mulch for insect, disease, and weed control (*8*).

Studies have shown that typically 1 to 6% of applied agrochemicals are removed from agricultural areas due to surface runoff (*9*). However, polyethylene mulch is impermeable and significantly reduces rainfall infiltration leading to increased runoff volumes and soil erosion (*10-12*), and greater pesticide loads with runoff weeks after application (*10, 13*). Thus, the combined use of polyethylene mulch with copper hydroxide would be expected to negatively impact nearby ecosystems since elevated levels of copper have been shown to affect aquatic organisms adversely (*14-18*). This runs countercurrent to organic agriculture's primary goal of optimizing the health and productivity of the interdependent communities of soil life, plants, animals, and people (*1*).

The objective of this research was to compare the conventional polyethylene mulch management practice (POLY-Bare: polyethylene-covered beds with bare soil furrows) with two alternative management practices (POLY-Rye: polyethylene-covered beds with rye covered furrows; VETCH: hairy vetch residue mulch on beds and furrows) to evaluate their effectiveness in reducing soil erosion and concomitantly decreasing copper loads in runoff.

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Materials and Methods

Site Description and Management Practices

The study site was located at the Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland. The field, composed of Mattapex silt loam, had an average slope of 5.8% north to south and 2.6% east to west and was divided into sixteen, 162-m^2 plots that were prepared in a north-south direction. A randomized complete block design was used to assign eight plots to tomato production while the remaining eight plots were planted with corn. Tomato and corn plots were rotated annually to reduce pest pressure. Each tomato plot contained four raised beds and earthen berms to guide runoff from the three central furrows to a fiberglass H-flume. Runoff from the tomato plots was measured and collected using an automated flow meter and runoff sampler containing twenty-four 350-mL glass bottles (ISCO model 6700, Lincoln, NE, USA).

During the 1998 and 1999 field seasons, four tomato plots were assigned to each of the following treatments: 1) tomatoes grown on raised polyethylene-covered beds with bare soil furrows between the beds (POLY-Bare) or 2) tomatoes grown on raised beds with both beds and furrows covered with hairy vetch residue mulch (VETCH). In the 2000 and 2001 field seasons, four tomato plots were assigned to each of the following treatments: 1) POLY-Bare or 2) tomatoes grown on raised polyethylene-covered beds with cereal rye planted in the furrows between the beds (POLY-Rye). Kocide® 101 (Griffin Corporation, Valdosta, GA, USA), a fungicide and bactericide containing 77% copper hydroxide, was applied at recommended rates three or four times during the latter part of the growing season.[1] Additional site characteristics and management practice details, pesticide application schedules, and precipitation events are presented elsewhere (*13, 19*).

Precipitation Events and Runoff Collection

A tipping-bucket rain gage was used to measure the time and intensity of each precipitation event. ISCO 6700 automated runoff-samplers installed at the edge of each plot were equipped with a bubbler flow module (model 730). Each was programmed to collect samples on a flow-weighted (volume) basis. Individual and integrated water samples were characterized in terms of total suspended solids, total copper, or dissolved- and particulate-phase copper.

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Copper Extraction and Analysis

Runoff samples were filtered to separate the dissolved-phase (< 0.45 µm) from the particulate-phase (> 0.45 µm) copper. Dissolved-phase copper was extracted from the filtered water samples using standard nitric acid (HNO_3) and hydrochloric acid (HCl) digestion (*20*); recoveries from spiked samples were 96.9 ± 3.4%. Particulate-phase copper concentrations were determined by extracting particulates captured on filter papers with a diethylenetriamine-pentaacetic acid (DTPA) solution (*21*); recoveries of copper from spiked samples were 102.0 ± 6.8%. All samples were analyzed using a Varian SpectrAA 300/400 atomic absorption spectrophotometer (wavelength: 324.8 nm, flame: air acetylene); minimum instrumental detection limit for copper was 0.03 ± 0.01 µg/mL (*13, 19*).

Statistical Analysis

Analysis of variance (ANOVA) was performed comparing runoff collected during each of the field seasons. Least significant difference determined statistical significance between treatment means for each runoff event (*22*). The single criteria of classification for the data during the 1998 and 1999 field seasons were mulch treatment, POLY-Bare or VETCH, while the single criteria for data classification in the 2000 and 2001 field seasons were furrow treatment, POLY-Bare or POLY-Rye. Correlation analyses were performed to identify factors that had the greatest impact on dissolved- or particulate-phase copper loading.

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[1] Mention of specific products or supplies is for identification and does not imply endorsement by U.S. Department of Agriculture to the exclusion of other suitable products or suppliers.

Results and Discussion

Production

An important consideration for growers who want to adopt organic farming practices is the impact on harvest yields. Several researchers have reported previously on the positive economic feasibility and greater harvest yields of the VETCH management practice relative to the conventional management practice of POLY-Bare (average tomato yield for 1991 to 1996: POLY-Bare = 6,820 ± 2,010 g/m^2, VETCH = 8,690 ± 1,690 g/m^2) (*23, 24*). In this study, no significant difference in harvest yield was observed between the POLY-Bare and POLY-Rye management practices within each year (2000: POLY-Bare = 2,380 ± 630 g/m^2, POLY-Rye = 2,040 ± 600 g/m^2; 2001: POLY-Bare = 3,850 ± 965 g/m^2, POLY-Rye = 4,010 ± 1,400 g/m^2); however, 2001 was almost two times more productive as a function of cooler weather in 2000.

Runoff Volume

Runoff volume is influenced by the rate and quantity of rainfall and rainfall infiltration. The practice of covering raised tomato-beds with polyethylene mulch greatly reduces rainfall infiltration because 50 to 75% of the field is covered with an impermeable surface. McCall et al. (*11*) and Wan and El-Swaify (*12*) reported greater runoff volumes associated with the use of impermeable plastic mulch relative to bare soil.

POLY-Bare vs. VETCH

In this study, significantly (p = 0.05) larger volumes of runoff were collected from POLY-Bare than from VETCH plots in 94% and 92% of the events measured in 1998 and 1999, respectively. Plots with polyethylene mulch had up to thirty-four times more runoff than the vegetative mulch plots for individual storm events (*8, 11*). The seasonal water loss for the 1998 and 1999 growing seasons are presented in Figure 1A. The observed decrease in runoff

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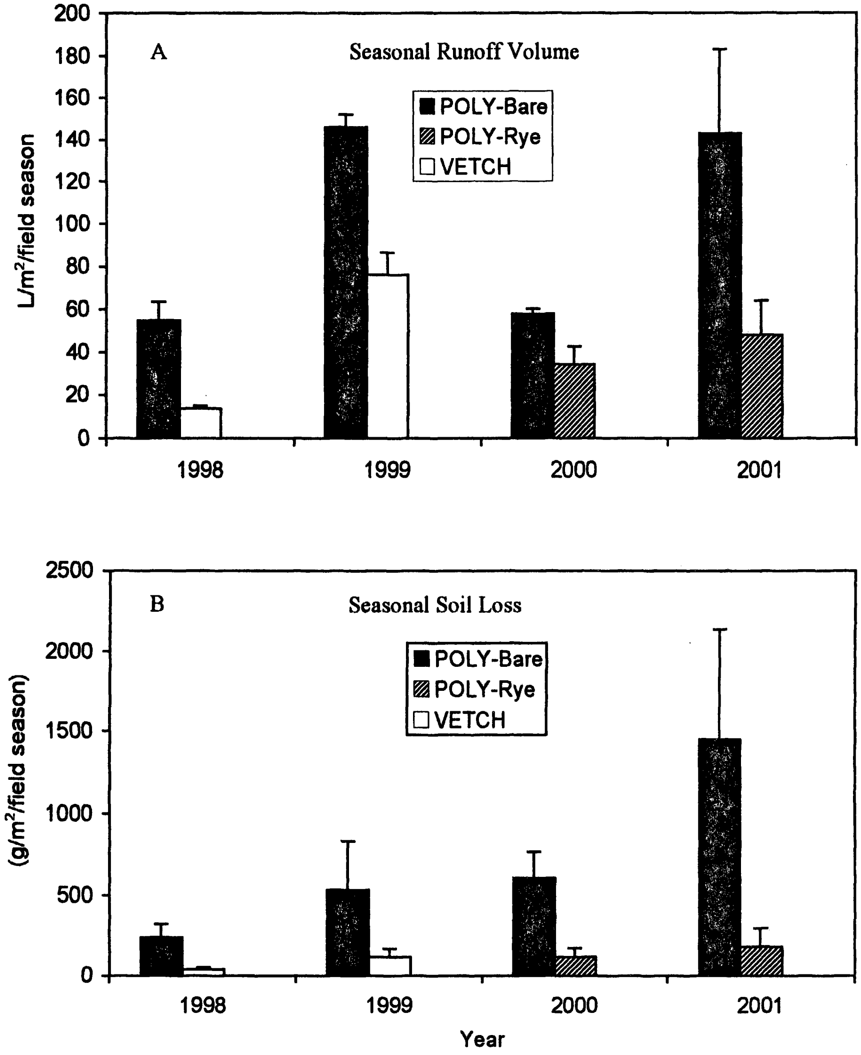

Figure 1. Seasonal runoff volume (A) and soil loss (B) from the polyethylene mulch/bare-soil furrow plots (POLY-Bare), polyethylene mulch/cereal rye furrows plots (POLY-Rye), and hairy vetch residue mulch plots (VETCH). Error bars represent the standard deviation of the mean.

volume (75% in 1998; 48% in 1999) for VETCH plots relative to the POLY-Bare plots is the result of the dissipation of rain drop energy and greater rainfall infiltration.

POLY-Bare vs. POLY-Rye

As expected, significantly (p = 0.05) larger volumes of runoff were also collected from POLY-Bare than POLY-Rye for 80% of the runoff events in 2000 and 62% of the runoff events in 2001 (*19*). Runoff volumes from plots with bare-soil furrows were up to eight times greater than the runoff volumes from the plots with vegetative furrows for individual storm events. Seasonal water losses for the 2000 and 2001 growing seasons are presented in Figure 1A. The runoff volume from POLY-Rye plots was reduced by 41% in 2000 and 66% in 2001 relative to the plots with bare soil furrows (POLY-Bare). This reduction may be due to increased water demand and reduced soil moisture associated with living vegetation in the furrows, changes in soil structure, and the ability of rye residues to dissipate the energy of rain drops and effectively reduce the velocity of surface runoff allowing for greater infiltration within the furrows (*25-28*).

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Soil Erosion

Soil loss (g/m^2) was calculated based on the total volume of runoff water collected per plot per runoff event, the mass of filterable suspended-solids per volume of runoff, and the size of each plot.

POLY-Bare vs. VETCH

The mean concentration (mg/L) of suspended-solids in runoff water from the POLY-Bare plots was four times greater than the concentrations measured in runoff from the VETCH plots (geometric mean for 1997, 1998 and 1999 = 3,330 mg/L for POLY-Bare plots and 692 mg/L for VETCH plots; range of individual runoff events for 1997, 1998, and 1999 = 424 to 16,810 mg/L for POLY-Bare plots and 12 to 6,952 mg/L for VETCH plots). When runoff volumes were considered, significantly (p = 0.01) greater loads of soil were lost with runoff from polyethylene mulch plots. Although the average soil losses for individual runoff events were up to 24 times greater from the POLY-Bare plots than the VETCH plots, the average soil losses for the 1998 and 1999 growing seasons were 6 and 4 times greater from polyethylene mulch than the vegetative residue mulch as shown in Figure 1B (*10, 13*).

POLY-Bare vs. POLY-Rye

Implementation of vegetative furrows in the polyethylene mulch production system significantly reduced the quantity of soil loading associated with runoff in 94% and 90% of the runoff events in 2000 and 2001, respectively. Runoff from POLY-Bare plots contained up to 24 times the concentration (g/mL) of suspended-solids than runoff from POLY-Rye plots with average particulate concentrations for POLY-Bare plots 4 and 5 times greater than from POLY-Rye plots (geometric means = 6,436 mg/L [2000] and 8,385 mg/L [2001] for POLY-Bare plots and 1,694 mg/L [2000] and 1,704 mg/L [2001] for POLY-Rye plots; range of individual runoff events for 2000 and 2001 = 353 to 18,642 mg/L for POLY-Bare plots and 84 to 5,225 mg/L for VETCH plots). The load of soil (g/m^2) measured in individual runoff events from the POLY-Bare plots was 2 to 44 times greater than the amount measured in the runoff from the plots with vegetative furrows. The average soil loss for the 2000 and 2001 growing seasons is presented in Figure 1B (*19*).

The greater runoff volume and flow rates associated with the POLY-Bare plots increased the opportunity for off-site transport of soil with runoff. Planting cereal rye between raised polyethylene-covered vegetable beds reduced the velocity of runoff flow by 1.3 to 2.4 times that of the POLY-Bare plots. Replacement of the polyethylene mulch with hairy vetch residue mulch further reduced runoff volumes with flow rates that were 1.2 and 7.5 times less than the runoff from the POLY-Bare plots. The significant reduction in soil loading with the two alternative management practices, containing either vegetative furrows or vegetative furrows and beds, is the result of the anchoring characteristics of plant roots providing increased structural stability of the vegetated soil and the ability of crop residues to dissipate the energy of raindrops and effectively reduce the velocity of surface runoff (*25-30*).

Copper Fate

Copper is present in both soluble and particulate forms in the environment. The chemical form of copper is important to its bioavailability, behavior in biological processes, and its toxicity to aquatic organisms. In aerated water with a pH range of most natural waters (6 to 8), cuprous copper (Cu^{+1}) is unstable and will oxidize to the cupric form (Cu^{+2}), which is the predominant oxidation state in soluble aqueous complexes and considered the most environmentally relevant and toxic form to aquatic life (*31-33*). Cupric ions may sorb to organic particulates, sediments and clays, and form complexes with organic or inorganic compounds (*34, 35*).

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Dissolved-phase Copper

Significantly (p = 0.01) greater loads of copper were measured in the dissolved-phase runoff from POLY-Bare plots (0.12-1.75 mg/m^2) than VETCH plots (0.06-0.84 mg/m^2) in all runoff events following the application of copper hydroxide (Figure 2A). At the completion of the field season, dissolved-phase copper loads represented 1.39% and 0.39% of the copper applied to the POLY-Bare plots and VETCH plots, respectively. Correlation analysis (r) revealed that runoff volume contributed more to the increased copper load than the concentration of copper measured in the filtered runoff water (POLY-Bare: r = 0.66 volume, r = 0.46 copper concentration; VETCH: r = 0.90 volume, r = 0.03 copper concentration).

Dissolved-phase copper loads were greater in the runoff from POLY-Bare plots (0.04 to 7.0 mg/m^2) than POLY-Rye plots (0.02 to 2.0 mg/m^2) for half of the runoff events following the application of copper hydroxide (Figure 3A). The seasonal load of copper measured in the dissolved-phase of the runoff was 2.4 times greater in plots with bare-soil furrows than vegetative furrows, which represented 2.1% and 0.9% of the applied copper, respectively. Correlation analysis indicated that the greater dissolved-phase loads of copper from plots with bare-soil furrows were the result of the increased runoff volume rather than a difference in dissolved-phase copper concentrations (correlation analysis (r): POLY-Bare: r = 0.93 volume, r = 0.002 copper concentration; POLY-Rye: r = 0.84 volume, r = 0.03 copper concentration).

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Particulate-phase Copper

Individual runoff events collected from POLY-Bare plots contained significantly (p = 0.05) greater loads of particulate-phase copper than runoff from VETCH plots (0.99-60.6 mg/m^2 for polyethylene; 0.06-8.65 mg/m^2 for hairy vetch) (Figure 2B). Copper loads in the particulate-phase of runoff represented 34.55% and 3.76% of the copper applied during the 1999 season for POLY-Bare plots and VETCH plots, respectively. Correlation analysis (r) of the initial runoff event following the application of copper hydroxide revealed that particulate-phase loads of copper were attributed more to the quantity of soil lost with runoff than the concentration of copper associated with the particulates (POLY-Bare: r = 0.88 soil loss, r = 0.00 copper concentration; VETCH: r = 0.75 soil loss, r = 0.19 copper concentration).

Runoff collected from POLY-Bare plots (0.8 to 136 mg/m^2 per runoff event) contained two to five times greater loads of particulate-phase copper than runoff from POLY-Rye plots (0.3 to 28 mg/m^2 per runoff event) (Figure 3B). Seasonal copper loads measured in the particulate-phase of runoff were 32.6% (POLY-Bare) and 8.8% (POLY-Rye) of the copper applied during the 2001

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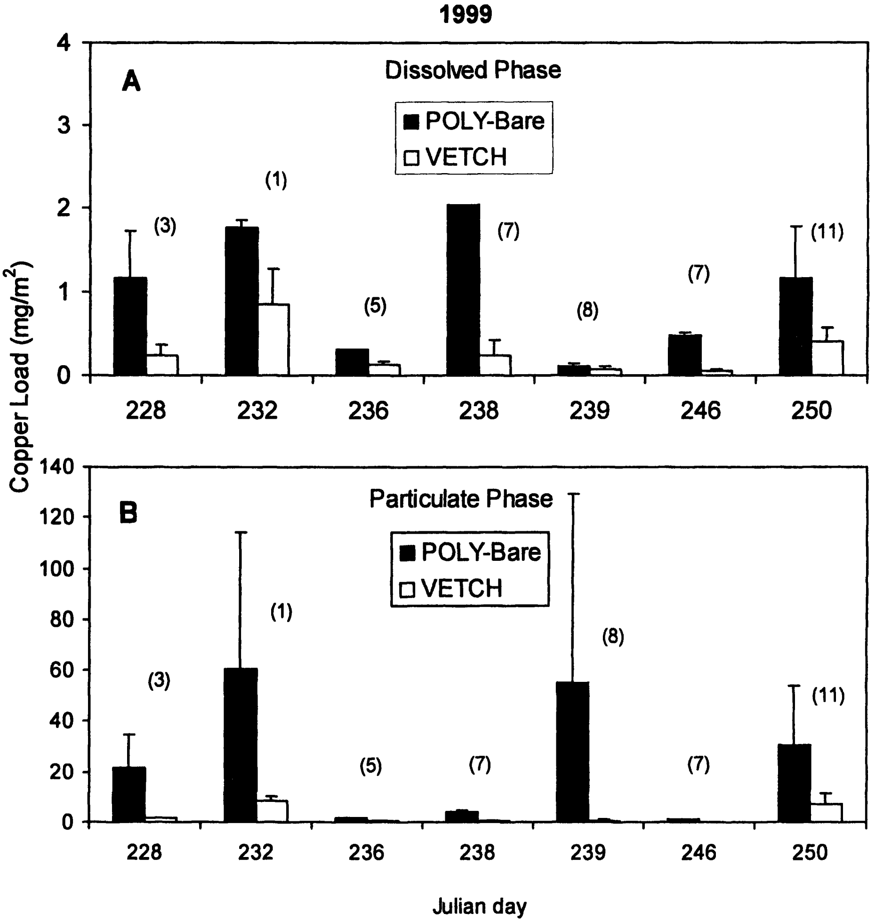

Figure 2. Dissolved-phase (A) and particulate-phase (B) loads of copper in the runoff from POLY-Bare and VETCH plots. Error bars represent the standard deviation of the mean. Numbers in parentheses represent days between copper hydroxide application and runoff.

growing season. Suspended-particles from the POLY-Rye plots contained greater concentrations of copper than particulates from POLY-Bare plots. However, correlation analysis (*r*) of runoff events following the application of copper hydroxide showed that particulate-phase loads of copper were attributed more to the quantity of soil lost with runoff than the concentration of copper on the particulates (POLY-Bare: $r = 0.98$ soil loss, $r = 0.002$ copper concentration; POLY-Rye = 0.99 soil loss, $r = 0.06$ copper concentration).

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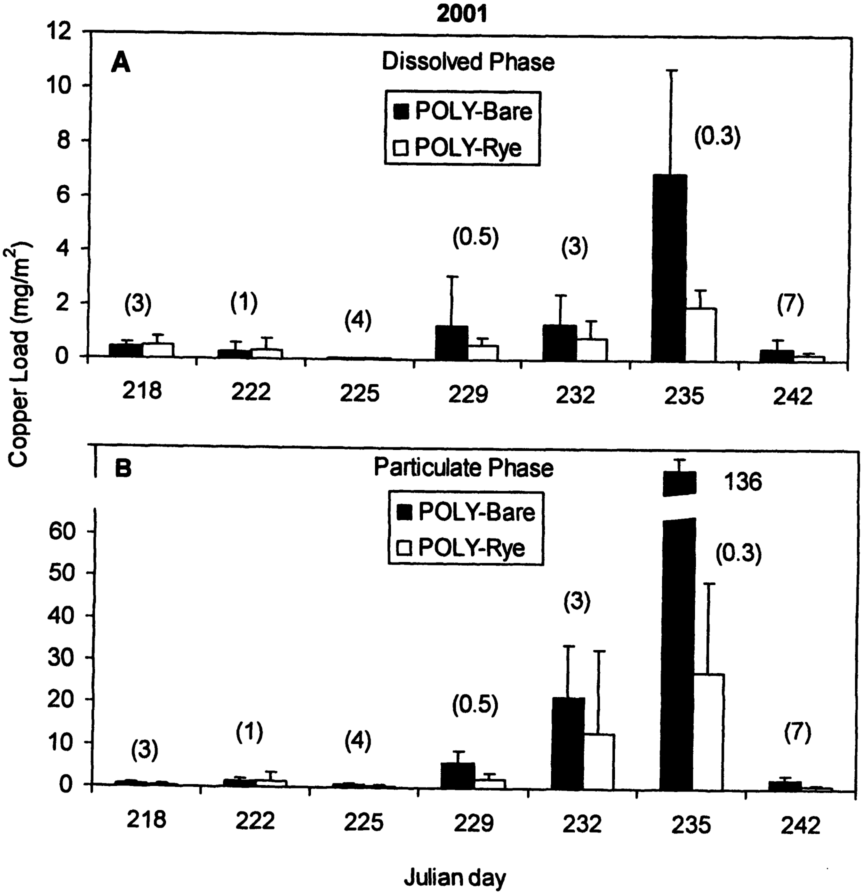

Figure 3. Dissolved-phase (A) and particulate-phase (B) loads of copper in the runoff from POLY-Bare and POLY-Rye plots. The difference in copper loads between the two management practices was significant (p=0.05) for runoff events on Julian day 229, 232, 235, and 242.

Phase Distribution

Although copper hydroxide is applied to the tomato plants, inevitably, a percentage of this fungicide is either washed off the foliage onto the mulch or directly applied to the mulch during foliar application. Copper is readily washed off polyethylene mulch and transported in surface runoff long after application (>30 days post application) (*13*). Furthermore, over 80% of the copper load was measured in the particulate-phase of the runoff for the three management practices (POLY-Bare = 86.7 ± 14.3%, 93 ± 6.5%; POLY-Rye = 88 ± 11%; VETCH = 81.7 ± 13.0%) (Figure 2A & 2B, Figure 3A & 3B). The impervious nature of polyethylene mulch produces greater volumes of runoff with larger sediment loads (*10-12*), resulting in greater off-site transport of copper from the area of application. Thus, controlling soil losses in runoff is critical to decreasing total copper released from the field.

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Ecotoxicological Concerns

Runoff from tomato production with polyethylene mulch has been implicated in the failure of commercial shellfish farms in the Mid-Atlantic Region of the United States. When freshwater containing copper-sorbed particulates comes in contact with seawater in estuarine environments copper is desorbed from the particulates as soluble copper (*36, 37*), which is more bioavailable to aquatic organisms. Aquatic biota may bioconcentrate copper in their tissue (bioconcentration factors: 28,200 for saltwater bivalves, 2,000 for freshwater algae) (*32*) and copper is acutely toxic to aquatic species at low levels (1.3 μg/L for *Daphnia* tested in freshwater, 1.2 μg/L for a bivalve tested in saltwater) (*14-18*). Copper has been shown to adversely affect fish, causing histological alterations in chemoreceptors, mechanoreceptors, and gill, kidney, and hematopoietic tissues (*17, 18*), and result in reproductive effects such as reduced egg production and abnormalities in newly hatched fry (*18*).

Dietrich et al. (*37*) reported that copper levels in a tidal creek receiving runoff from an agricultural area utilizing polyethylene mulch (POLY-Bare), were as high as 22 μg/L, which exceeds the measured median lethal concentration (LC_{50}) for larval clams (*Mercenaria mercenaria*) at 96 h (LC_{50} = 21 μg/L) and 192 h (LC_{50} = 12 μg/L). Based on the results of our field investigations, implementation of alternative management practices that either maintain the polyethylene mulch but replace bare soil furrows with vegetative furrows (POLY-Rye) or completely replace the impermeable polyethylene mulch with vegetative residue mulch (VETCH) will reduce copper loads with runoff by an average of 72% and 88 %, respectively. Therefore, surface water concentrations could be reduced from 22 μg/L to approximately 6 and 3 μg/L, which are below the LC_{50} for larval clams, assuming agricultural runoff was the primary source of copper. These reduced concentrations are also near or below the EPA guidelines

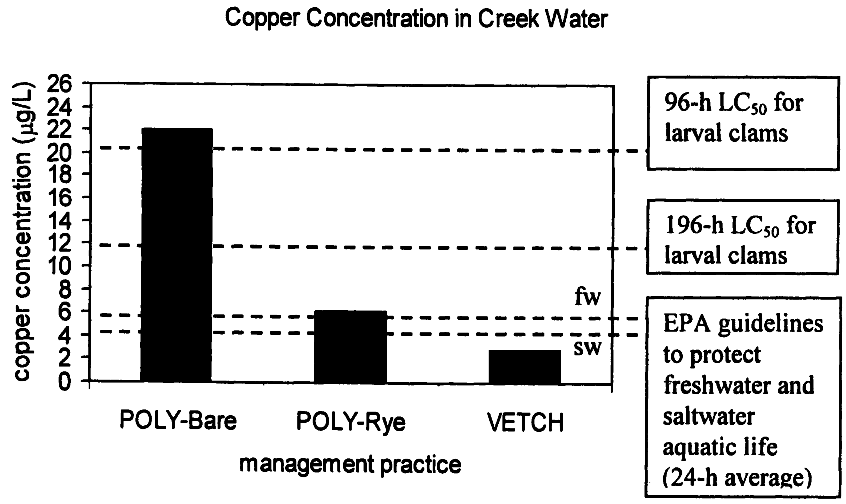

Figure 4. Impact of agricultural management practices on the concentration of copper in a creek receiving runoff from fresh-market vegetable production. fw = freshwater, sw = saltwater.

(*31*) to protect freshwater and saltwater aquatic life (24-h average = 5.4 μg /L for fresh water and 4.0 μg /L for salt water) (Figure 4).

Conclusion

The results of this study have clearly demonstrated that management practices can have a profound impact on agrochemical fate, even if both the cultivation method and the chemical are allowed under NOP standards. The chemical characteristics and toxicity of copper-based products require that they be used in a judicious and environmentally-friendly manner to avoid negative impacts on surrounding ecosystems.

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Chapter 18

A Review of the Environmental Fate and Effects of Natural "Reduced-Risk" Pesticides in Canada

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Bioactive compounds derived from microbial, plant, or other natural sources are a largely untapped source of new pesticides. They are also widely considered to have characteristics conferring reduced risk to the environment and a high potential for use in modern integrated pest management strategies. In examining the "reduced-risk" hypothesis, the fundamental physico-chemical properties, mechanisms of dissipation and laboratory toxicity data for technical active ingredients phosphinothricin, azadirachtin, and spinosad were assessed. Hazard quotient analysis, which relates expected environmental concentrations to laboratory toxicity data, indicated little cause for concern in terms of predicted environmental fate but potential toxicological risks for certain non-target species such as bees, zooplankton, and aquatic plants. Environmental fate and ecotoxicological effects data for the derivative natural product pesticide formulations Ignite® and Herbiace®, Neemix® 4.5 and Success®, as derived from Canadian field studies, were also summarized. Results from the field studies generally confirm the hazard quotient risk analysis and demonstrate substantial ecotoxicological risks for formulated products based on phosphinothricin and azadirachtin active ingredients, particularly in freshwater aquatic ecosystems. Based on these evaluations, and in comparison to reference synthetic pesticides glyphosate and tebufenozide, we find no evidence to support the hypothesis that natural products pose inherently lower risk to the environment than these synthetic pesticides. While we fully support further research and development of natural product pesticides, we suggest that these or any other pest control product must be fully and comprehensively evaluated through a tiered research and environmental risk assessment process, culminating in controlled field studies, environmental monitoring and probabilistic risk analysis.

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Several factors including public demand, legislative pressures, and the scientific search for compounds with novel modes of action, have been key in stimulating research and development of pesticides with reduced risk to human or environmental health. Both the U.S. EPA and the Canadian Pest Management Regulatory Agency (PMRA) have designed regulatory programs to define criteria for classifying pesticides as "reduced-risk" and for encouraging their development and registration (*1*). Under this harmonized system, numerous factors are considered in the decision to classify a pesticide as having reduced risk. Compounds with a high degree of efficacy, use patterns which may displace chemicals of human health concern (e.g., probable carcinogens) and those with relatively greater selectivity, lower mammalian toxicity, lower potential for non-target effects or lower potential for pest-resistance buildup, may be considered favorably. In addition, compounds exhibiting low potential for movement or persistence in the environment and those involving lower use rates or fewer applications may be selected. In general, the intent is to encourage development of pesticides that are efficacious, environmentally acceptable and compatible with modern integrated strategies for pest management.

Naturally occurring bioactive compounds derived from microbial, higher plant or other natural sources have been viewed as virtually untapped sources of potential new pesticides (*2-4*). They are also considered as potentially useful components of modern integrated pest management strategies (*5,6*). Throughout the scientific and regulatory literature, substances derived from natural sources and showing substantial pesticidal activity have been variously referred to as biorationals (*7-9*), biopesticides (*10,11*) or natural pesticides (*2,3,12-14*). The multiplicity of terms, combined with lack of clear definition and inconsistent use, creates considerable confusion, particularly among lay audiences. Moreover, a misperception, particularly widespread among the general public and environmental activist groups, but which also appears in the scientific literature (*15*), holds that "natural" products are inherently safer or more environmentally acceptable than synthetic compounds. This may not necessarily be true, and the zeal with which scientists and corporations promote research and development of natural product pesticides may unwittingly promulgate this misperception.

With regard to the reduced-risk concept, many natural compounds do tend to exhibit novel modes of action, high water solubility, facile metabolism and low mammalian toxicity; however this is also true for most modern synthetic pesticides. The environmental fate and toxicity of natural pesticides are controlled by the same fundamental physical, chemical, biological and toxicological principals that govern the fate and effects of synthetic pesticides. Thus, there is little fundamental rationale for distinguishing between natural and modern synthetic pesticides in terms of their environmental acceptability. The diversity of chemical structures and fundamental physico-chemical properties that make natural compounds attractive as potential sources of new pesticides also assures that they will exhibit a wide variety of environmental and toxicological behaviors. Aflatoxins, botulinus toxin, and ricin are all natural products characterized by exceedingly high mammalian toxicity and classic examples that refute the validity of the "natural is better" generalization. Thus, while there is clear value in screening natural products for useful biological activities, rational development of these products requires investigation and assessment of environmental persistence, fate, and toxicological properties, with a scientific rigor equivalent to that applied to any other pest control agent – synthetic or natural, biological, or chemical.

In this paper, we review the current knowledge base available in the peer-reviewed scientific literature as it pertains to the environmental fate and potential effects of natural pesticides based on the active ingredients phosphinothricin, azadirachtin, and spinosad (Figures 1-3). The focus of this manuscript is in relation to registered or potential use in Canada (Table I), particularly in forest management scenarios to which the bulk of the data is most pertinent. In comparison to the reference synthetic pesticides glyphosate and tebufenozide, we also assess the data in relation to the postulate of "reduced-risk" as it pertains in the context of modern Canadian forest pest management. General concepts apply more broadly, and recommendations for further research on the environmental fate and effects of the natural pesticides presented are not specific to any particular use pattern.

Phosphinothricin-Based Herbicides

Phosphinothricin is a natural metabolite derived from the actinomycete *Streptomyces viridochromogenes* or *S. hygroscopicus* (*16*) and the common active ingredient of both a natural herbicide commonly referred to as bialaphos (bilanophos) and a synthetic derivative referred to as glufosinate-ammonium (Figure 1). The mode of action and basic toxicology of glufosinate-ammonium

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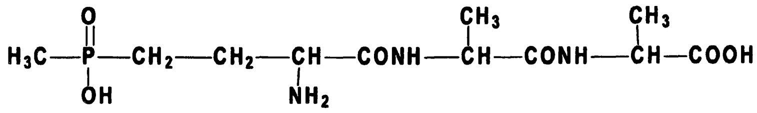
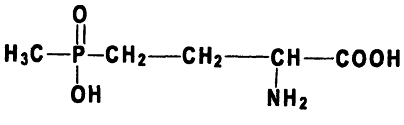


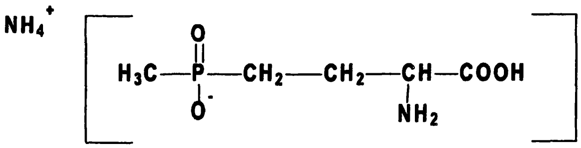
$H_3C-P(=O)(OH)-CH_2-CH_2-CH(NH_2)-CONH-CH(CH_3)-CONH-CH(CH_3)-COOH$

BIALAPHOS



$H_3C-P(=O)(OH)-CH_2-CH_2-CH(NH_2)-COOH$

PHOSPHINOTHRICIN



$NH_4^+ \left[H_3C-P(=O)(O^-)-CH_2-CH_2-CH(NH_2)-COOH \right]$

GLUFOSINATE AMMONIUM

Figure 1. Chemical structures of phosphinothricin, bialaphos and glufosinate ammonium, the active ingredients of phosphinothricin-based herbicides such as Herbiace®, Finale®, Ignite® or Liberty®

has been previously reviewed by Hoerlein (*17*). Phosphinothricin exhibits potent phytotoxicity to a wide variety of plant species through inhibition of the glutamine synthetase enzyme (*18,19*). Several commercial products containing phosphinothricin from either biogenic (e.g. Herbiace® Meija Seika Kaisha Ltd., Tokyo, Japan), or synthetic origin (e.g. Finale® AgrEvo Canada Inc., Regina, Saskatchewan; Ignite® Bayer Crop Science Inc., Research Triangle Park, NC) have been registered in various countries for total vegetation control in non-cropland, orchards and vineyards, as a crop-dessicant or, most recently, in transgenic crops. In Canada and the United States, a phosphinothricin-based formulation (Liberty® Bayer Crop Science Inc., Research Triangle Park, NC) has been registered for use on phosphinothricin-resistant crops including canola, soybeans and corn. Phosphinothricin-resistant forest crop species have also been the subject of significant research and development initiatives as described below.

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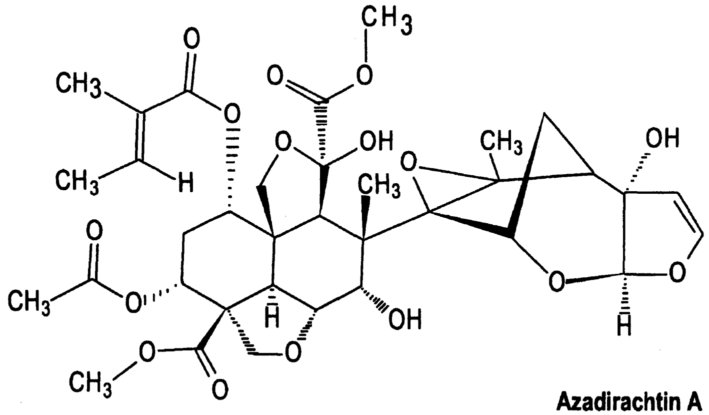
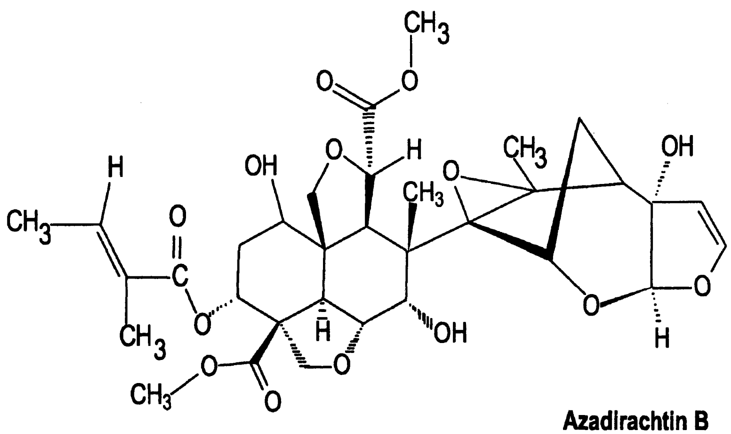


Azadirachtin A



Azadirachtin B

Figure 2. Chemical structures of azadirachtins A and B, the active components of azadirachtin-based insecticides such as Neemix® 4.5

Although several studies were undertaken to examine the potential for use of phosphinothricin-based herbicides in Canadian forest vegetation management (*20-24*), no attempt to register a product for this use pattern has been made owing to a demonstrable lack of tolerance for major coniferous crops (*24*) and concerns regarding potential environmental effects in aquatic systems as described below. The recent development of several transgenic tree species with resistance to glufosinate (*25-29*) demonstrates a potential mechanism for overcoming the crop-tolerance problem but raises other potential concerns regarding introgression of trans genes into wild tree populations (K. van Frankenhuysen personal communication; *29*).

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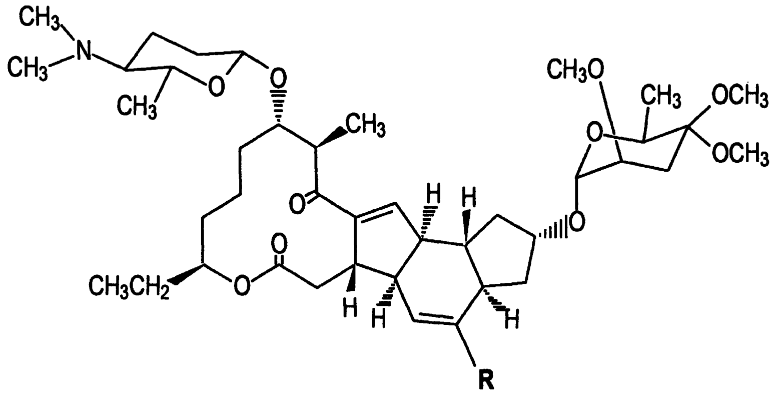

Spinosyn A: R = H Spinosyn D: R = CH_3

Figure 3. Chemical structures of spinosyns A and D, the active components of spinosad-based insecticides such as Success® and Conserve®

The fundamental physico-chemical properties controlling the environmental fate and behaviour of phosphinothricin are summarized in Table II. High water solubility and low log K_{OW} values suggest that phosphinothricin may be mobile in soils but is unlikely to bioaccumulate. As a highly polar, weak-acid with three dissociable hydroxyl and amino moieties, phosphinothricin has three pKa values. Under normal environmental pH it occurs predominantly in its ionized state, and therefore readily forms salts and is non-volatile. Laboratory studies demonstrate that several soil bacteria are capable of degrading phosphinothricin and utilizing it as a source of nitrogen (*30,31*). Thus, environmental dissipation is largely mediated through aerobic microbial biotransformation (*30*). Under laboratory conditions, phosphinothricin degrades rapidly in soils with time to 50% dissipation (DT50) ranging from 3 to 19 days, depending upon soil type and temperature (*32*). The principal metabolites formed via microbial degradation are the 2- or 3-methyl phosphinyl propionic acids (MPPA 2 & 3) (*32*) which exhibit characteristics of high water solubility, low Kow and susceptibility to microbial degradation similar to the parent compound. Smith (33) studied the laboratory degradation of phosphinothricin in Canadian prairie soils at 10°C and 20°C and reported DT50 values of 3-7 and 8-11 days, respectively, demonstrating that microbial degradation of phosphinothricin is temperature-dependent. Over a 90-day incubation period at 20°C, between 28 and 55% of the applied radioactivity was released from treated soils as carbon

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Table I. Natural and Reference Pesticides With Registered or Potential Use in Canada

Natural Product	*Commercial Formulations in Canada*	*Active Ingredient Concentration*	*Mode of Action*	*Target Pests*	*Use Patterns*	*Canadian Registration Status*
Phosphinothricin (glufosinate ammonium)	Finale 1SN & 6SN	60 g L^{-1}	Inhibits glutamine synthesis	All non-resistant weedy vegetation	resistant corn and canola;	
	Ignite	137 g L^{-1}				
	Wipe Out	105 g kg^{-1}		Vegetation	non-crop & desiccant	
Azadirachtin	Neemix 4.5	41 g L^{-1}	molt disruption	Balsam fir sawfly; yellow-headed spruce sawfly; pine false webworm	forestry	Temporary (Sect 17) Oct 2000
Spinosad	Success 480SC (NAF-85)	480 g L^{-1}	persistent activation of nicotinic acetylcholine receptors	oblique banded leaf roller; spotted tentiform leafminer; sod webworm; elm leaf beetle; sawfly; gypsy moth; eastern tent caterpillar	apples turf ornamentals	Temporary (Sect 17) Aug 2001
	Conserve 480SC (Tracer)	480 g L^{-1}				
Glyphosate (reference herbicide)	Roundup	356 g L^{-1}	inhibits aromatic amino acid synthesis	all non-resistant weedy vegetation	resistant corn & soybeans;	
	Vision	356 g L^{-1}		competing grass; herbaceous and deciduous brush	non crop dessicant; forestry	Registered 1984
Tebufenozide (reference insecticide)	Mimic	240 g L^{-1}	ecdysone agonist	spruce budworm; eastern hemlock looper,	forestry	Registered 1996
	Confirm			codling moth; oblique-banded leafrollers; tentiform leafminer	Apples	Registered 1996

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

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Table II. Physicochemical Properties of Technical Active Ingredients for Natural Pesticides and Reference Synthetic Pesticides

Natural Product	*Water Sol.*[a]	*log K_{OW}*[b]	*K_{OC}*[c]	*pKa*[d]	*VP*[e] *(kPa)*	*λ_{max}*[f]	*$T_{1/2}$ (hydrolysis)*[g]	*$T_{1/2}$ (photolysis)*[h]	*$T_{1/2}$ (microbial)*[i]	*Primary Dissipation Mechanisms*	*Primary Metabolite*
Phosphinothricin	1370	-1		<2 2.9 9.8	nil				3-19	microbial	MPPA 2 & 3 oxidative decarboxylation deaminination
Azadirachtin	0.05	1.1	5.1-7.9	none	0.285	220	pH=4 19 pH=7 13 pH=10 < 1	4 <1-7 (on plants)	6-26 20 - 44	hydrolysis, photolysis, microbial	di-& tetra-hydro azadirachtins hydroxylation
Spinosyns A & D	235	4.0	844-1.4 x10^5	8.10	3.0 x10^{-11}	244	pH=5 nil pH=7 nil	1-2 82 (soil)	9-17	photolysis microbial	demethylated spinosyns A/D
	0.332	4.5	672-7.8 x 10^4	7.87	2.0 x 10^{-11}	244	pH=9 200/259		15 44 (D)		demethylation
Glyphosate (reference herbicide)	12000	-3.2	3.0 x 10^4	2.3 5.6 10.3	nil		nil	<14	<60	microbial photolysis	AMPA decarboxylation
Tebufenozide (reference insecticide)	0.83	4.2	351-894	none	3 x10^{-6}	234	pH=5 568 pH=7 1034 pH=9 517	98	105-704	microbial photolysis	several alkyl oxidation

Note: Data were compiled from a variety of different sources including Canadian regulatory documents (*55*, *130*, *133*) and various other published studies as referenced in the text.

a) Water Sol. = solubility in neutral water; units of µg mL^{-1} @ 20-25 °C
b) log K_{OW} = logarithm of the n-octanol:water partition coefficient at neutral pH; log $K_{OW} > 3$ raises potential concerns re: bioaccumulation
c) K_{OC} = organic carbon partition coefficient
d) pKa = acid dissociation constant
e) VP = vapour pressure in units of (mm Hg @ 20 °C)
f) λ_{max} = wavelength of maximal absorption in the ultraviolet-visible range
g) $T_{1/2}$ (hydro) = time in days required for 50% degradation by hydrolysis in pond water at 20 °C
h) $T_{1/2}$ (photo) = time in days required for 50% degradation by photolysis in water except where indicated
i) $T_{1/2}$ (biotrans) = time in days required for 50% degradation by soil microbes under aerobic conditions at 10-25 °C

dioxide. Laboratory studies show that degradation products may be temporarily incorporated into the microbial biomass but ultimately degraded to CO_2 (*17*). Behrendt et al. (*34*) reported a DT50 for the MPPA metabolite of 30 to 50 days and indicated it would be substantially more persistent and mobile than the parent compound. The bialaphos molecule occurs in products derived from fermentation reactions and is rapidly converted to phosphinothricin (*15*) in soils. Phosphinothricin undergoes further degradation in soils with a reported DT50 value of 20 to 30 days.

Standard laboratory toxicity test endpoints (Table III) indicate that phosphinothricin is relatively non-toxic to mammals, birds, fish, algae, or zooplankton following acute exposures at realistic concentrations. Not surprisingly, aquatic plants (*Lemna paucicostata; Lemna gibba, and Myriophyllum sibiricum*) with EC50 values as low as 0.30 mg L^{-1} (*35*), appear to be the most sensitive non-target organisms. No information was found on the toxicity of technical phosphinothricin to earthworms or bees, but a reported earthworm LC_{50} for the Basta® formulation (200 g a.i. L^{-1}) of ≥1000 mg kg^{-1} suggests no significant toxicity to these organisms. In laboratory studies, Ahmad et al. (*36*) demonstrated that phosphinothricin may influence soil microbial community structure with significant inhibitory effects on *Bacillus subtilis* and *Pseudomonas fluorescens* both of which are antagonistic to pathogenic fungi.

Relatively few standard toxicity data are available for bialaphos; however, Mase (*15*) reported an LD50 value of 5000 mg kg^{-1} for white leghorn chickens and LC50 values of 1000 mg L^{-1} for *Daphnia*. The same author reported that technical bialaphos was much less toxic to carp (LC50 = 1000 mg L^{-1}) as compared to the formulated product Herbiace® (6.8 mg L^{-1}).

Several Canadian field studies have documented the environmental fate of phosphinothricin in agricultural and forestry soils. Studies conducted in a variety of agricultural soils demonstrate that phosphinothricin is neither persistent nor susceptible to leaching (*37-40*). Reported DT50 values for the parent compound range from 3-11 days in all studies. Field studies also indicate that phosphinothricin rarely leaches below 10-15 cm in agricultural soils (*38-41*). Faber et al. (*41*) investigated the degradation and leaching potential of phosphinothricin, following application of ^{14}C glufosinate ammonium to a northern Ontario forest soil. Results demonstrated rapid dissipation of phoshphinothrin (DT50 = 4.3 days) and formation of the principal MPPA metabolites, which also degraded over time. No leaching below the 10 cm humic layer was observed for either phosphinothricin or its metabolites.

The fate and effects of the phosphinothricin-based herbicides produced by fermentation reaction (Herbiace®; bialaphos) and chemical synthesis (Ignite®; glufosinate ammonium) were compared following applications to in-situ enclosures deployed in a forest pond (*35*, *42*) in northern Ontario, Canada. Dissociation of the glufosinate-ammonium salt resulted in rapid formation of

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Table III. Comparative Acute or Sub-Acute Toxicity Values for Technical Grade Natural Pesticides and Reference Synthetic Pesticides as Determined in Standard Laboratory Studies

Active Ingredient	*Mammals*[a]	*Birds*[a]	*Bees*[b]	*Earthworms*[c]	*Fish*[c]	*Zooplankton*[c]	*Algae*[d]	*Aquatic Plants*[d]
Phosphinothricin[e]	1510	?1000			?320	?560	?37	0.3
Azadirachtin	3540	> 255	>2.5		0.048	0.39		0.04
Spinosad	>2000		0.0025	970	5.9 30	92.7	106	1.86 (NOEC)
Glyphosate (reference)	5600	3800	>100	119	10	780	22	10
Tebufenozide (reference)	>5000	2150	234	1000	>100	17.37 (>0.83 water sol. limit)	0.16	

[a] For mammals and birds, values are actue oral LD50 (mg a.i. kg^{-1} body weight)

[b] For bees (*Apis mellifera*), values are 48-h contact LD50, expressed in units of ug a.i. per bee

[c] For earthworms (*Eisenia foetida)*, fish (*Onchorhynchus mykiss*), aquatic plants (*Lemna* spp) and zooplankton (*Daphnia magna*) values are 48-96 h LC_{50} in mg a.i. kg^{-1} soil or mg a.i. L^{-1} water

[d] For algae (*Selenastrum capricornutum*), values are LC50 based on the concentration in mg a.i. L^{-1} water required to inhibit growth by 50% relative to controls over an exposure period of 7 days

[e] Lowest reported data taken from Hoerlein (*17*), except mammalian actue oral LD50 and aquatic plant LC50 taken from Faber et al. (*35*)

phosphinothricin in enclosures treated with Ignite®, followed by slow exponential decline with an estimated DT50 of 43-63 days depending upon initial concentration. In contrast, the bialaphos molecule degraded at a moderate rate (DT50 = 12 to 15 days) forming phosphinothricin in the process. Thus, in enclosures treated with Herbiace®, phosphinothricin accumulated over time following a hyperbolic saturation function, with maximal concentrations occurring at the last sampling prior to freeze-up.

Transient, concentration-dependent declines were observed in phytoplankton populations as well as in dissolved oxygen concentrations in response to both treatments. EC50 values for reduction in phytoplankton abundance were similar, ranging from 2.5 to 3.4 mg a.i. L^{-1} for Ignite® and 3.3 to 8.1 mg a.i. L^{-1} for Herbiace®. Differential recovery was observed with phytoplankton abundances returning to control levels within 14 days following exposure to high concentrations (10 mg a.i. L^{-1}) of Herbiace® as compared to 49 days following exposure to similar concentrations of Ignite®. At environmentally realistic concentrations, only minor impacts on the phytoplankton community were observed. However, both herbicides also induced substantial concentration-dependent effects on the abundance of several zooplankton taxa and the total zooplankton population. EC50 estimates ranged from 0.12 to 0.5 mg a.i. L^{-1}, well below worst-case expected environmental concentrations (EEC) of 1.05 mg a.i. L^{-1} as calculated by Canadian regulatory authorities (Table IV). Concentrations eliciting a response in zooplankton equivalent to the EC50 were considered to be well within the realm of exposures that might realistically occur through accidental direct overspray or drift. The severity of impacts observed in natural zooplankton communities, including species of Copepoda, Rotifera, and Cladocera, was not predictable based on standard laboratory toxicity data, suggesting that *Daphnia* spp. are relatively insensitive to both technical and formulated products (*32*). This discrepancy indicated that other zooplankton taxa are particularly sensitive to phosphinothricin, that the two formulations tested were substantially more toxic than those examined in previous literature studies, or that the natural zooplankton community was responding to multiple stressors including depressed oxygen and reduced algal food resources induced by the treatments.

The effects of phophinothricin (Herbiace®) and tebufenozide (MIMIC®) on zooplankton community structure were subsequently compared by ordination of species assemblages using principle components and correspondence analysis (*43*). Results demonstrated clear, concentration-dependent effects of phosphinothricin while the effects of tebufenozide were considered equivocal. The persistent and significant impacts observed on zooplankton abundance and community structure under typical environmental conditions and realistic exposure concentrations, resulted in the conclusion that substantial mitigative measures would be required to protect against phosphinothricin impacts on natural zooplankton communities.

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Table IV. Expected Environmental Concentrations

Natural Product	*Commercial Formulation*	*MLR*	*EEC Soil*[a]	*EEC Water*[b]	*EEC Foliage*[c]
Phosphinothricin	Ignite	1.5	0.66	1.05	1848
Azadirachtin	Neemix 4.5	0.05	0.02	0.04	62
Spinosad	Success, Conserve	0.26	0.09	0.17	322
Glyphosate (reference herbicide)	Vision	2.14	0.94	1.43	2636
Tebufenozide (reference insecticide)	Mimic	0.07	0.03	0.05	86

MLR – maximum label rate expressed in units of kg a.i. ha^{-1}

[a] EEC_{Soil} - values expressed in units of mg a.i. kg^{-1}; calculated for tier I risk analysis assuming a soil bulk density of 1.5 g/cm 3 and uniform distribution of the compound throughout a soil depth of 15 cm, at the maximum label rate to the bare soil; for spinosyns the calculation assumes 3 consecutive sprays 7 days apart

[b] EEC_{Water} – values expressed in units of mg a.i. L^{-1}; calculated for tier I risk analysis assuming full deposition at the maximum label rate to a body of water 15 cm in depth

[c] $EEC_{foliage}$ – values expressed in units of mg a.i. kg^{-1} fresh weight; calculated for tier I risk analysis assuming full deposition at the maximum label rate to leaves and leafy crops on the day of application, as determined using a standard U.S. EPA nomogram

Azadirachtin-Based Insecticides

The azadirachtins are a family of natural tetranortriterpenoid compounds derived from seeds of the neem tree (*Azadricacta indica* A. Juss. [Meliaceae]) and the putative active principles in a wide variety of natural insecticide formulations (*44*). As noted by several authors (*44,45*), neem seed extracts contain a variety of active tetranortriterpenoid compounds including seven isomers (AZA-A through -G) of azadirachtin. Among these isomers, azadirachtin A reportedly constitutes 85% of the neem seed extract and has shown the greatest insecticidal activity (*44,46*) (Figure 2). Although the exact

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mechanism of action is incompletely understood, azadirachtins are known to disrupt insect molting as controlled by the 20-hydroxyecdysone hormone (*47*) and to exhibit strong antifeedent, growth regulation and reproductive effects (*44, 46, 48*). The azadirachtins, particularly AZA-A, are characterized by substantial insecticidal activity on a variety of insects of importance to both forestry and agriculture. In Canadian forestry applications, azadirachtin-based pesticides have been shown to be effective against spruce budworm (*Choristoneura fumiferana* (Clemens)); gypsy moth (*Lymantria dispar* L.); birch leafminer (*Fenusa pusilla* (Lepeletier)); balsam fir sawfly (*Neodiprion abietis* (Havris)); and pine false webworm (*Acantholyda erythrocephala* (L.)) (*49-54*). In Canada, the commercial formulation Neemix 4.5® (Thermo Trilogy Corp., Columbia, MD) was granted temporary registration for aerial applications against sawfly pests in Canadian forestry (*55*). However, that registration has now lapsed. In this regard, an Australian review and assessment of toxicological literature associated with various products derived from the neem tree (*56,57*) has garnered significant regulatory attention and may influence further research and development of azadirachtin-based products in Canada.

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The physico-chemical properties of technical azadirachtin (Table II) indicate low water solubility, low bioaccumulation poential, and low volatility. Relatively short half-life estimates for hydrolysis, photolysis and biotransformation as estimated from laboratory studies indicate that azadrichtins are susceptible to several dissipation mechanisms including hydrolysis, photolysis and aerobic biotransformation. Hydrolysis is known to be strongly base-catalyzed with half-life of 2 hours at pH 10 as compared to 19.2 days at pH 4 (*58*). Azadirachtin A (λ_{max} = 220 nm) is susceptible to photolysis with a photolytic half-life for pure azadirachtin A approximating 4 days, and a similar value of 7 days estimated for azadirachtin on plant surfaces (*59*). One or more of the photodegradation products are at least as biologically active as the native molecules (*59*).

Low K_{OC} and K_{OW} values suggest that azadirachtins are only moderately sorbed to organic materials and thus may be susceptible to leaching in soils. Leaching column and adsorption/desorption studies conducted by Sundaram (*60*) confirm that sorption of AZA-A to sandy loam soils is limited and reversible. Laboratory studies suggest that the persistence of azadirachtin in soils is dependent on soil characteristics as well as temperature (DT50 26-44 days) (*61,62*) and that it is also non-persistent on treated foliage (DT50 < 1 day) (*45,63*). Szeto and Wan (*64*) and Sundaram (*45*) have also demonstrated temperature-dependent, base-catalyzed hydrolysis of AZA-A as the dominant mechanism of dissipation in natural waters. Hydrolysis of formulated products is significantly slower than that of pure technical AZA-A (*45*).

Standard laboratory toxicity test endpoints (Table III) support the conclusion of studies with insect natural enemies (*65*) that azadirachtin is relatively non-toxic to mammals, birds, and bees. Studies by Stark and Walter (*62*) suggest that azadirachtins also have a relatively narrow spectrum of activity with low toxicity to non-target and beneficial organisms as compared to conventional insecticides. Kreutzweiser (*66*) examined the acute lethal effects of 2 azadirachtin-based pesticide formulations on 8 different species of aquatic macroinvertebrates in flow-through screening tests. Significant mortality was recorded only in one mayfly species (*Isonychia bicolor*) with an estimated LC50 value for Azatin of 1.12 mg a.i. L^{-1} which is about 30 X the EEC. Neither formulation caused significant mortality or antifeedant effects to three aquatic detritivore insects after a 28-day exposure at the EEC of 0.035 mg L^{-1} azadirachtin. Stark (*67*) recently demonstrated that, although technical azadirachtin shows relatively little toxicity to *Daphnia pulex* (LC50 = 13 mg L^{-1}), formulated products may be substantially more toxic (Neemix and Azatin LC50 = 0.68 and 0.57 mg L^{-1}, respectively). The author concluded that because the NOEC for population growth and reproduction was higher than the estimated environmental concentration of 0.035 mg L^{-1}, Neemix® 4.5 should pose little risk to populations of *D. pulex*.

The aquatic fate and effects of azadirachtin have been examined in a variety of Canadian field studies. Using outdoor stream channels, Kreutzweiser et al. (*68*) examined potential community level effects on stream insects resulting from short-term (5-h) exposures to Neemix® 4.5. Only 1 of 8 taxa showed a significant behavioral (drift) response at the maximum test concentration of 0.84 mg L^{-1}. The survival rates of *Isogenoides sp.*, *Isonychia bicolor* and *Hydropsyche bifida* were significantly reduced by exposure to 0.84 mg L^{-1} of azadirachtin applied as Neemix® 4.5. However, no effect on mortality was observed for the latter 2 species when exposed to azadirachtin concentrations of 0.28 mg L^{-1}. In further stream channel experiments, there were significant differences in aquatic insect community structure between controls and channels treated at 0.84 mg L^{-1}', but not between controls and channels treated at 0.28 mg L^{-1} of azadirachtin. The formulation ingredients of Neemix® 4.5 were at least partially responsible for the significant effects on community structure at 0.84 mg L^{-1} (*69*). The concentration of 0.28 mg L^{-1} azadirachtin, at which no significant effects on stream insect communities were detected, was about 8 times the EEC. It was concluded that aquatic insects typical of those found in forest streams are not particularly sensitive to azadirachtin or the formulated product Neemix® 4.5

Scott and Kaushik (*70*) also found that two applications of azadirachtin applied as the formulated product Margosan-O® did not harm aquatic invertebrates categorized as planktonic and filter feeding (*Culex* sp. and *Daphnia* sp.). However, the benthic invertebrate (*Chironomus riparius*) was affected by multiple applications of neem and the authors concluded that

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Margosan-O® and possibly other azadirachtin formulations could lead to disturbances in aquatic benthic invertebrate populations or nutrient cycling processes. Dunkel and Richards (*71*) found that aquatic invertebrates were adversely affected at environmentally realistic concentrations when testing with an Align® formulation, but noted that petroleum-based components of Align® increased the toxicity of azadirachtin to aquatic insects in their bioassays. Stark and Walter (*62*) demonstrated that neem oil and other limonoid and polar compounds contained therein significantly affected the toxicological response of *Acyrthosiphon pisum.* This result suggests that the toxicity of formulations derived from neem seed extracts cannot be unilaterally ascribed to the putative active principals azadirachtin A and B and is likely to depend on variations in the concentration of other bioactive substances which may differ with various methods of extraction and purification used to generate technical products. To our knowledge, there have been no systematic studies assessing the contribution of other bioactive substances to the toxic effects observed in sensitive target and non-target organisms.

The fate and potential effects of azadirachtin in forest ponds have been extensively examined using in-situ enclosures (*72-76*). These studies demonstrate that azadirachtin A dissipates from the water column at moderate rates following linear kinetics with little influence of natural seasonal variations in dissolved organic carbon, temperature, water color or sunlight irradiation and with little sorption to either suspended or bottom sediments. Estimated DT50 values of 25-29 days as observed by Thompson et al. (*72*) in a forest pond with depth ~ 0.5 m and average pH ~ 5.6 differed significantly from values of 1.5-2 days previously reported for Neem-EC and Margosan-O formulations applied to small outdoor microcosms (*70,77*). Differences in the observed dissipation rates could not be unequivocally attributed to any single factor since pH, temperature, irradiation (depth and intensity) as well as formulations tested differed among the various studies and each factor has previously been demonstrated to influence azadirachtin dissipation rates (*45,64*). However, it is likely that the dissipation rates observed by Scott and Kaushik (*70*) were enhanced by increased rates of photolysis in the shallow water depth (10 cm) in their outdoor test systems.

Assessment of zooplankton impacts indicated that the azadirachtin formulation Neemix® 4.5 caused significant, concentration-dependent reductions in adult copepods at and above the EEC of 0.035 mg azadirachtin L^{-1} (Table IV), while cladocerans and rotifers were not affected at the EEC (69). In a subsequent study of the same formulated product (*75*), even lower azadirachtin concentrations of 0.01, 0.017, and 0.028 mg L^{-1} resulted in significant concentration-dependent reductions in adult copepods, but immature copepod and cladoceran populations were unaffected. No evidence of recovery of adult

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copepods was observed within the sampling season (May to October). The ecological significance of this disturbance to the zooplankton community was examined by determining biomass as a measure of food availability for higher predators; plankton community respiration, dissolved oxygen concentrations, and conductivity as functional indicators of ecosystem stress; and zooplankton food web stability as a measure of effects on trophic structure. The selective removal or reduction of adult copepods was sufficient to measurably reduce total zooplankton biomass for several weeks mid-season. During the period of maximal impact (about 4 to 9 weeks after the applications), total plankton community respiration was significantly reduced, and this appeared to contribute to significant concentration-dependent increases in dissolved oxygen and decreases in conductivity among treated enclosures. The reduction in numbers of adult copepods resulted in negative effects on zooplankton food web stability through eliminations of a trophic link and reduced interactions and connectance (*76*).

In relation to terrestrial fate and effects, Thompson et al. (*78*) recently reported on the deposition of azadirachtin A following aerial applications of Fortune AZA 3% in red pine plantations where mean foliar concentrations (0.44 to 1.47 μg g^{-1} fresh weight) were well in excess of concentrations (0.29 ± 0.07 μg g-1 fresh weight) generating 91% mortality in laboratory bioassays against the target insect pine false webworm. Lyons et al. (*79*) confirmed the efficacy against this insect pest in parallel field studies.

Several completed and ongoing studies have investigated the fate and effects of azadirachtin in plants following systemic injections to various deciduous and coniferous crop species. Sundaram et al. (*80*) demonstrated that azadirachtin was readily taken up by aspen (*Populus tremuloides*), with measurable concentrations in various plant tissues within 3 days following applications to the soil. At 10 days post-treatment highest residues on a fresh weight basis were observed in the roots (173 μg g^{-1}), stems (50 μg g^{-1}) and foliage (22 μg g^{-1}). Ongoing studies (Thompson et al. unpublished, *80,81*) indicate that azadriacthin is rapidly taken up and translocated following systemic injections to a variety of deciduous and coniferous tree species. A number of studies also show substantive efficacy on a variety of target insect pests following systemic trunk injections.

To our knowledge there have been no field studies documenting the fate of azadirachtin in soils. However, studies conducted under laboratory conditions resulted in estimated DT50 values ranging from 20 to 115 days depending on the isomer (A or B), temperature, and whether soils were autoclaved to reduce microbial activity (82). In these studies, low temperature and low microbial activity enhanced persistence of both isomers and AZA-B tended to be more persistent than AZA-A under all test conditions.

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Spinosyn-Based Insecticides

The spinosyns are a family of macrolide substances isolated from the actinomycete *Saccharopolyspora spinosa*, and include at least twenty-three different compounds with insecticidal activity (*83*). The technical active ingredient in commercial formulations, spinosad, is defined as the sum of spinosyns A and D (Figure 3). Spinosyns are known to persistently stimulate the central nervous system of insects through interaction with the nicotinic acetylcholine receptors. This mechanism of action is considered distinct from other nicotinic agonists (*84*). Insecticidal activity has been demonstrated against several pests of economic importance in agriculture and forestry. Spinosad-based formulations have been developed for insect pest control in numerous crops including cotton, vegetables, and fruits as well as in ornamental trees and turf in both the United States and Canada. In Canada, the first spinosad formulations (Success® 480SC and Conserve® 480SC) were registered for use on apples, outdoor ornamentals and turf in 2001. Although not currently registered for use in Canadian forestry, substantial efficacy against various forest pests including gypsy moth (*85,86*) as well as favourable environmental fate results from terrestrial studies conducted in two key forest environments (*87,88*) support continued research and development for this potential use pattern.

An examination of key physico-chemical properties (Table II) demonstrates that spinosyns A and D exhibit substantially different water solubility. The relatively high log K_{OW} ($\geq$ 4) and K_{OC} values suggest that both substances partition into lipids and bind to soil organic matter. Based on their pKa values, spinosyns A and D would be expected to occur in both ionized and molecular form at environmentally relevant pH, with relatively greater proportions in the ionized state under more alkaline conditions. Degradation of spinosad in the environment occurs principally through photolysis and microbial degradation (*89*). Sorption maxima and very short photolysis half-life values (Table II) show that spinosad is highly susceptible to photolysis, and to a lesser extent, degradation by hydrolysis and aerobic biotransformation. Aerobic soil biotransformation rates have been shown to be somewhat temperature-dependent, with greater persistence observed at lower temperatures. The principal pathway of degradation is via N-mono-demethylation of the forosamine sugar moieties of both spinosyn A and D (*90*) and demethylation reactions are known to have substantial effects on insecticidal activity (*91*).

Spinosad shows minimal acute oral toxicity (rat LD50 rats > 5000 mg kg-1) and is non-carcinogenic, non-teratogenic, non-mutagenic and non-neurotoxic in mammalian test animals. Spinosad also has low toxicity to several beneficial hemipteran, coleopteran, and neuropteran insects as well as beneficial acarina (*91*). The product shows relatively low acute toxicity to fish, algae (*S. capricornutum*), zooplankton (*D. magna*) and earthworms. A recent

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comparative laboratory study (*92*), using a population demographics approach and the zooplankton species *D. pulex*, indicated that spinosad was significantly less toxic than diazinon.

In laboratory studies, high acute contact toxicity has been observed with bees (LC50 of 0.0025 µg a.i. per bee). Substantial chronic toxicity has been shown with aquatic midge larvae (25 day NOEC = 0.0014 mg a.i L^{-1}) and the diatom *Navicula pelliculosa* (120-h NOEC = 0.049 mg a.i L^{-1}). As we have noted in previous publications (*93,94*), laboratory toxicity studies in general, and particularly those involving labile compounds, may tend to over-estimate environmental exposures and therefore potential effects.

Comprehensive risk assessment often requires a tiered approach culminating in concomitant assessment of pesticide fate and effects under scenarios relevant to proposed use patterns. Where extensive data on environmentally relevant exposure regimes and toxicity to numerous species exist, probabilistic assessments (e.g. *95*) may also be employed. Either approach may yield results that support or refute the postulate of risk as derived from simple, single-species lab toxicity studies alone. In this regard, a rapidly expanding knowledge-base is available on the potential non-target effects of spinosad-containing products, including two recent reviews (*96,97*), a comprehensive ecological risk assessment (*98*), and numerous papers summarizing specific laboratory or field studies (*99-112*). The existing knowledge base is dominated by laboratory studies and focused largely on potential effects to bees and other terrestrial non-target predators and parasitoids, particularly as these relate to cotton production scenarios.

The persistence and activity of both the active ingredient and degradation products are important factors controlling the potential for non-target effects. For this reason, exposure to dried or "aged" residues have been used in some studies in an effort to enhance environmental realism while maintaining experimental control. Determination of the residual fate and bioactivity of spinosad is complicated (*113*) and may be assumed to depend to some degree on environmental factors controlling the primary photolytic or microbial degradation pathways. In at least one case (*114*), spinosad residual activity has been demonstrated for periods of up to 28 days post-application. This finding contradicts the assertion of Williams et al. (*97*) that all studies on spinosad demonstrate little residual activity at 3-7 days post-application. Overall, it appears that there is no weight-of-scientific evidence that tips the scale to either side of the debate. Moreover, extrapolation to all possible use patterns cannot be supported given the highly case-specific nature of the available data.

Few field studies have been conducted on the environmental fate and effects of spinosad relative to potential use patterns in Canada. Thompson et al. (*87,88*) conducted studies in both Ontario and New Brunswick examining the persistence and leaching of spinosad following application of the NAF-85 formulation to graminaceous thatch, coniferous tree litter, and mineral soils.

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Consistent with their high Koc values, neither spinosyn A nor D showed any potential for leaching. DT50 values for spinosyn A ranged from 2.0 to 12.4 days depending upon microsite and matrix condition, while spinosyn D dissipated to below quantitation limits within 7 days in all cases. Sporadic low-level detection of the demethylated metabolites of spinosyn A and D suggested that parent compounds were degraded in situ. Shorter dissipation times (<1 day) reported for agricultural soil studies conducted in the United States may reflect differences in temperature, photolysis rates, or microbial populations. To our knowledge, there have been no studies published on the fate of spinosad on foliage or in aquatic ecosystems pertinent to potential use patterns in Canada.

Assessment of Natural Pesticides as "Reduced-Risk" for Canadian Forest Pest Management

Progressive evolution towards more environmentally benign pest control products requires comparative assessment of the fundamental physico-chemical properties, environmental fate and potential toxicological effects of both the technical active ingredients and formulated products in relation to other commonly used products for a specific use pattern.

In relation to their potential use in Canadian forest pest management, natural products considered in this review have been compared to glyphosate and tebufenozide which are the most common synthetic pesticides used in Canadian forest management. In Canada, glyphosate, formulated as the isoproylamine salt and chemically equivalent to Roundup®, represents more than 90% of the forest herbicide use-market (*115*). The environmental fate and potential toxicological effects of technical glyphosate and its formulated products have been extensively studied and recently reviewed (*95,116*). Similarly, tebufenozide sold as the commercial formulation MIMIC® is the only synthetic insecticide currently with significant use in Canadian forestry. A substantial environmental fate and effects knowledge base derived from laboratory and field studies is also available for tebufenozide (e.g. *117-120*).

Relative to the reference herbicide glyphosate, phosphinothricin-based herbicides offer an alternate mode of action, a high degree of efficacy, moderately lower use rates and thus lower expected environmental concentrations (Table IV). However in terms of fundamental physico-chemical properties (Table II) and environmental fate (Table V), phosphinothricin-based herbicides do not differ significantly from the reference herbicide glyphosate.

Comparison of standard laboratory test endpoints for the technical active ingredients suggests that phosphinothricin has lower or equivalent toxicity to fish, algae and *Daphnia* spp., but is substantially more toxic to aquatic plants (*Lemna* spp.) and somewhat more toxic to mammals as compared to glyphosate

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Table V. Comparative Fate of Natural Pesticides Based on Canadian Field Research

Natural Product	*Commercial Formulation*	*DT_{50} Soil (d)*	*DT_{50} Water (d)*	*DT_{50} Foliage (d)*	*Leaching Potential*	*Refs.*
Phosphinothricin	Ignite	4.3	43-63		not significant	*35, 41*
Azadirachtin	Neemix 4.5	< 1	26-29 1.5 1.5-2	5-7	mobile (lab soil column leaching study)	*45, 70, 72, 77*
Spinosad	Success, Conserve	2-12		7.8	not significant	*87, 88*
Glyphosate (reference herbicide)	Vision	10-12	4-26	2	not significant	*126, 136, 137*
Tebufenozide (reference insecticide)	Mimic	31-68	32-35	30-59	not significant	*77, 134, 138*

(Table III). Laboratory studies also demonstrate that phosphinothricin-based herbicides may influence soil microbial populations whereas several studies indicate that this is not the case for environmentally realistic concentrations of glyphosate (*121-124*). Finally, field ecotoxicology studies demonstrate that phosphinothricin-based herbicides formulated as either Ignite® (glufosinate ammonium) or Herbiace® (bialaphos) generate significant and sustained effects on zooplankton communities in forest ponds. Glyphosate, on the other hand, has been shown to have minimal effects on zooplankton in a forest pond (*125,126*) or on algae of forest ponds and streams (*127-129*). Based on these comparisons there is little scientific basis to conclude that phosphinothricin-based herbicides present a reduced risk relative to the industry standard glyphosate.

Azadirachtin and tebufenozide have similar maximum use rates in Canadian forest insect pest management and thus EECs in various matrices are approximately equivalent. Neither azadirachtin nor its reference compound tebufenozide are highly soluble in water; however azadirachtin shows a substantially lower propensity to partition to organic materials including organic

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carbon in soils and is therefore more susceptible to leaching (Table II). Both products are characterized by low vapor pressures and thus have little potential to volatilize from treated surfaces. Azadirachtin is relatively more susceptible to hydrolysis, photolysis and aerobic biotransformation (Table IV) and, as such, is markedly less persistent than tebufenozide in all environmental compartments (Table V). Regulatory concerns have been expressed regarding the persistence of tebufenozide, particularly in aquatic sediments (*130*).

Comparative laboratory toxicity data for technical active ingredients are lacking in some aspects (Table III). Although neither azadirachtin or tebufenozide is acutely toxic to mammals or birds, azadirachtin may be more toxic to fish but results have been variable. Some have demonstrated LC50s to fish of well over 1 mg L^{-1} (*131,132*), while others have reported LC50s of <0.05 mg L^{-1} (55). Field ecotoxicology studies have demonstrated substantial potential for the formulated product Neemix 4.5 at azadirachtin concentrations well below the EEC to adversely affect zooplankton communities through selective effects on copepods. Potential effects of tebufenozide on zooplankton have also been raised as a concern (*130*). However, the no-observable effect value 0.029 mg ai. L^{-1} for *D. magna* (*130*) is similar to the EEC for forestry applications, and ecotoxicology studies have provided contrasting results. Following applications of tebufenozide (Mimic®) at concentrations up to 3 times greater than the EEC, no significant effects on zooplankton were observed (*134*) whereas a similar study conducted in a different aquatic system showed significant but relatively short-term effects on cladocerans at concentrations 2 times greater than the EEC (*135*). Thus, while azadirachtin compares favorably with tebufenozide in terms of persistence, it is potentially more susceptible to leaching and at least one formulated product has been repeatedly shown to generate significant and sustained effects on zooplankton communities in field ecotoxicology studies at or below the EEC. Overall, these comparisons do not support an unequivocal conclusion that azadirachtin represents a reduced risk for environmental effects in forest use scenarios as compared to the synthetic insecticide tebufenozide.

Comparative assessments of the environmental fate and effects of spinosad relative to tebufenozide are significantly constrained by a lack of field studies documenting environmental fate and potential effects for spinosad in aquatic systems. Spinosad is used at rates approximately 3 fold higher than those for tebufenozide, yielding higher values for expected concentrations in various environmental compartments. While several physico-chemical properties for these two compounds are similar, spinosad is substantially more susceptible to photolysis and aerobic biotransformation (Table II), leading to reduced persistence in soils and foliage as demonstrated in comparable Canadian field studies (Table V). Comparative laboratory toxicity data (Table III) demonstrate that spinosad is substantially more toxic to bees and somewhat more toxic to fish as compared to tebufenozide. In this case, there is insufficient scientific

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evidence to determine whether or not spinosad represents a reduced risk relative to tebufenozide in Canadian forest pest management scenarios.

Knowledge Gaps and Recommendations for Further Research

As one might expect given their relatively recent introduction, the scientific knowledge base on environmental fate and effects in Canadian environments for natural products based on phosphinothricin, azadirachtin and spinosad are generally limited in comparison to synthetic pesticides. A comparative summary of knowledge gaps pertinent to ecotoxicology in Canada is provided in Table VI.

One of the key differences between natural and synthetic pesticides is the potential for other biological active substances to occur in extracts from plant materials or fermentation products. Detailed analytical chemistry and bioassay guided fractionation studies are required to determine the occurrence and toxicity of such compounds as well as their potential additive or synergistic effects in determination of overall toxicity to representative target and non-target species. Further information that might be useful for detailed assessment of environmental fate and effects of these natural pesticides in Canada is listed below. It is recognized that some of this research may already be underway or available in proprietary submissions to regulatory agencies.

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Phosphinothricin-Based Herbicides

- Fate of phosphinothricin in treated foliage and ultimate fate of phosphinothricin foliar residues
- Laboratory and field studies on toxicity to bees, earthworms and amphibians
- Interactive effects of pH on toxicity of phosphinothricin to soil and aquatic organisms
- Potential fate and ecotoxicological implications of repetitive uses in either agricultural or forestry scenarios associated with phosphinothricin resistant crops
- Further laboratory and particularly field studies investigating the influence of phosphinothricin and degradation products on microbial community structure and function

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Table VI. Summary of Canadian Field Studies or Risk Assessments Indicating Significant (Yes) or No Significant (No) Effects Under Realistic Exposure Scenarios

Active Ingredient ***TEST FORMULATION***	*Soil Organisms*	*Birds*	*Bees*	*Fish*	*Algae*	*Zoo-plankton*	*Amphibians*	*Stream Insects*	*Refs.*
Phosphinothricin **IGNITE**	N/A	N/A	N/A	N/A	Yes	Yes	N/A	N/A	*35, 42*
Azadirachtin **NEEMIX 4.5**	No	No	No	Equiv	No	Yes	N/A	No	*55, 68, 69, 75, 132, 139*
Spinosad **SUCCESS, CONSERVE**	No	No	Yes	No	No	Yes	N/A	Yes	*133*
Glyphosate **VISION**	No	No	No	No	No	No	No	No	*94, 95, 125-129, 140, 142, 143*
Tebufenozide **MIMIC**	No	No	No	No	No	Equiv.	No	No	*118, 120, 130, 134, 135, 141*

Note: N/A indicates no Canadian field studies or risk assessments currently available

Azadirachtin-Based Insecticides

- Field assessment of leaching potential, particularly in sandy, coarse textured soils
- Translocation and fate of azadirachtin following systemic injection in key tree species
- Toxicity of azadirachtin residues in foliage to detritivores
- Fate and toxicological significance of degradation products
- Laboratory and field studies on toxicity to earthworms and amphibians
- Laboratory and field studies investigating the influence of azadirachtin and degradation products on soil and aquatic microbial community structure and function

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Spinosyn-Based Insecticides

- Aquatic fate and effects with particular emphasis on systems with reduced photolytic potential, persistence in sediments and potential impacts on sediment-dwelling organisms
- Field studies under typical use scenarios involving concomitant studies on fate, persistence and biological activity of spinosad and degradation products in relation to potential lethal/sublethal effects on terrestrial non-target organisms
- Laboratory and field studies investigating the influence of spinosad and degradation products on soil microbial community structure and function

Conclusions

Several bioactive compounds derived from microbial, plant or other natural sources have potential for use as pesticides in Canadian agriculture, forestry or non-crop scenarios. Natural pesticides are widely considered to have characteristics conferring reduced risk to the environment and a high potential for use in modern integrated pest management strategies. The fundamental physico-chemical properties, mechanisms of dissipation, laboratory toxicity and field fate and effects data for products based on phosphinothricin, azadirachtin and spinosad were assessed in comparison to reference synthetic pesticides glyphosate and tebufenozide. Based on these comparative evaluations, we find no evidence to support the hypothesis that natural products pose inherently lower risk to the environment. While we fully support further research and development of natural product pesticides, we suggest that these or any other pest control product or approach, must be fully and comprehensively evaluated through a tiered research and environmental risk assessment process, culminating in controlled field studies, environmental monitoring and probabilistic risk analysis.

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Chapter 19

Ecotoxicology of Neem

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Because pesticides derived from the neem tree are natural products, there is an automatic assumption that they are environmentally benign. However, pesticides by definition kill living things and because the primary active ingredient of neem, azadirachtin, affects the universal molting hormone of arthropods, neem pesticides should have negative effects on at least some nontarget arthropods. Many researchers have now evaluated the toxicity of neem pesticides on various nontarget organisms in the laboratory and field. In addition, several studies on the persistence of the primary active ingredient, azadirachtin have been published. Here the ecotoxicity of neem pesticides is reviewed focusing particularly on recent studies. The general conclusion is that neem pesticides are less damaging to nontarget organisms than certain synthetic pesticides. However, some nontarget organisms are particularly sensitive to neem pesticides and therefore as with all pesticides, they should be used with caution and continue to be evaluated for nontarget effects.

In Crop Protection Products for Organic Agriculture; Felsot, A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2006.

Pesticides derived from the neem tree are often considered to be environmentally safe and less damaging to ecosystems than synthetic pesticides. However, recent evidence indicates that neem products can cause damage to some nontarget organisms. There are many biologically active components in neem seed kernels and leaves, but the primary insecticidal component is the highly oxidized limonoid, azadirachtin (*1,2*). Other limonoids are also present in neem seeds that are toxic or repellent to insects (*3,4*). Azadirachtin works by inhibiting the release of morphogenetic peptide hormones resulting in the disruption of ecdysteroid and juvenile hormone concentrations in the hemolymph affecting molting, metamorphosis, and reproduction (*2*).

Many studies have been published on the effects of neem on nontarget organisms. It is not my intention to review all of the literature on the toxicity of neem to various organisms as this has been done several times in the past. For a comprehensive review of neem side effects on nontarget organisms see Schmutterer (*4*). Instead, the focus of this review will be on several more recent papers that deal with the effects of neem on nontarget organisms as well as field studies that involve an examination of the impact of neem on nontarget organisms.

Most of the published studies dealing with the effects of neem-derived pesticides on nontarget organisms concern biological control agents because the authors were looking for compatibility of these two control methods for integrated pest management. In general, neem pesticides appear to be less toxic to biocontrol agents compared to pest species (*4*) and appear to be less toxic than neurotoxic insecticides (*5,6*). The low toxicity of neem to biological control agents may be due to the requirement of oral ingestion, low toxicity to adult insects, systemic activity, short environmental persistence, and antifeedant and repellent properties (*3,7*). However, the universal molting hormone of arthropods is ecdysone and because azadirachtin interferes with this hormone, organisms such as crustaceans, spiders and predatory mites may be susceptible. In fact, certain species of biocontrol agents are quite susceptible to neem as discussed below.

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Toxicity of the Formulation Versus Toxicity of Neem Ingredients

A major problem exists with the interpretation of the neem literature. In many of the published studies dealing with the effects of neem pesticides on pest and beneficial species, very different neem extracts/forumulations, different seed sources, and extraction methods were used. Isman et al. (*8*) found that active ingredients vary greatly among different seed batches. As such, it is difficult to tell what active ingredients or lack of active ingredients were actually present in these formulations. With the advent of standardized commercial neem formulations and advances in analytical methodology for neem, many of the

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recent studies with neem pesticides are conducted with standardized formulations, making interpretation of the data much easier than in the past.

Several studies have been conducted that indicate that at least part of the toxicity observed in various species is due to the formulation of commercial neem products and not solely to the active ingredients. Sauke and Schmutterer (*9*) determined that the formulation of a neem insecticide and not the active ingredients were responsible for the toxicity observed in the water flea, *Daphnia magna*. Stark and Walter (*10*) found that neem oil accounted for 60% of the toxicity to pea aphids while Kreutzweiser et al. (*11*) suggested that the formulation accounts for some of the toxicity to aquatic invertebrates. Additionally, Stark (*12*) exposed *Daphnia pulex* to the commercial neem pesticide, Neemix and a formulation blank containing no neem components and found that 47% of the observed toxicity was due to the formulation. These results indicate that the toxicity attributed to neem may be due at least partially to the formulation.

Persistence of Azadirachtin

The persistence of azadirachtin, the primary insecticide in neem has been studied in soil, water and on foliage. Azadirachtin is as persistent as the carbamates carbaryl and methomyl and the pyrethroids esfenvalerate and permethrin in water and soil (*6*). Stark and Walter (*13*) found that azadirachtin had a disappearance time of approximately 20 days in soil at 25°C. This increased to 31-42 days when the soil was autoclaved indicating that microbes played a major role in degradation of azadiracthin. In water, azadirachtin had a 1-12 day half-life (14). Azadirachtin had a half-life on foliage of less than 1 day similarly to diazinon and malathion (*15*). Thus, the persistence of azadirachtin is similar to that of some synthetic insecticides (*6*).

Effects on Biocontrol Agents

Parasitoids

Many studies have been conducted on the effects of various neem products to parasitoids. Results of some studies indicate that neem pesticides are not very toxic to parasitoids. For example, injection of azadirachtin into tobacco budworm (*Manduca sexta* [Linnaeus]) larvae that had been parasitized by the braconid wasp *Cotesia congregata* (Say) did not negatively affect parasitoid development if the compound was administered after the parasitoids had ecdysed to the second instar (*15*). However, results of other studies have indicated that neem may indeed have detrimental effects on some parasitoid species. Stark et al. (*17*) examined the effects of a neem insecticide on three braconid parasitoids of tephritid fruit flies. Two of these parasitoids developed normally in flies that had been exposed to concentrations of azadirachtin that completely inhibited host fly eclosion. However, in one species, *Psytallia incisi* (Silvestri), reproduction was reduced 63-88% after exposure to 20 mg azadirachtin/L.

Laboratory experiments were carried out to study the effect of several different neem insecticides on *Trichogramma japonicum* Ashmead (*18*). The rate of parasitization and emergence of adults from parasitized eggs was examined. Econeem and Neem Azal were found to be safer compared to the synthetic insecticides quinalphos and chlorpyrifos, which had adverse effects on parasitization. However, the neem formulations Nimbecidine, Neemgold and Rakshak negatively affected parasitization.

Villanueva and Hoy (*19*) assessed the compatibility of several neem insecticides with the citrus leafminer parasitoid, *Ageniaspis citricola* (Logvinoskaya). They found that Neemix was compatible with this parasitoid, but Align and Neemgard were only semi-compatible for IPM of the leafminer. These differences in effect were probably due to differences in the type and amount of active ingredient and/or components of the formulations. The effects of neem seed oil on the egg parasitoid *Trichogramma chilonis* (Ishii) was determined by Raguraman and Singh (*20*). Oviposition deterrence was detected after exposure to neem oil concentrations of 0.3%.

Two neem insecticides were found to significantly reduce the food consumption of larvae and emergence of adult *Cotesia plutellae* (Kurdyumov), a parasitoid of the diamondback moth (*21*). Thakur and Pawar (*22*) found that the egg parasitoid *T. chilonis* was unaffected by two neem insecticides, Achook and Neemactin, while Goudegnon et al. (*23*) found no effect of neem extract on the parasitoid *C. plutellae*.

Akol et al. (*24*) investigated the effects of two neem insecticides on a parasitoid of the diamondback moth, *Diadegma mollipla* (Holmgren). They found that Neemroc and Neemros applied at rates that controlled the diamondback moth did not adversely affect survival and foraging behavior of *D. mollipla*. Matter et al. (*25*) studied the effects of neem on the *Pieris rapae* (Linnaeus) parasitoid, *Hyposoter ebeninus* (Grav). Treatment with neem at the LC50 level resulted in a large reduction in parasitoid progeny. However, treatment of *P. rapae* with low concentrations of neem (LC25) could reasonably potentiate parasitism 2-3 times over untreated hosts without drastic losses in parasitoid emergence. Tang et al. (*26*) evaluated the neem insecticide Neemix 4.5 as a control for the brown citrus aphid and examined the compatibility of this insecticide with a parasitoid of the aphid, *Lysiphlebus testaceipes* (Cresson). Parasitoid survival and development was virtually unaffected by Neemix.

The reproduction and survival of the egg parasitoid *Trichogramma minutum* Riley after exposure to two neem insecticides, Azatin EC and Neem EC, was

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evaluated by Lyons et al. (*27*). They established that exposure to 50 g azadirachtin/ha, resulted in no significant effect on female survival. However, exposure to 500 g azadirachtin/ha, significantly reduced female survival. Exposure to pure azadirachtin did not reduce survival, indicating that other components of the formulations were in part responsible for the toxicity to females.

The varying results of the studies listed above indicate that susceptibility of parasitoids to neem is dependent upon the species and the type of neem insecticide. No generality about the effects of neem pesticides on parasitoids can be drawn.

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Predators

Lady beetles

Adult seven spot lady beetles, *Coccinella septempunctata* L., were exposed to 2% neem seed kernel extract or 3% neem oil. Egg production was not affected by either treatment but metamorphosis was negatively affected (*28*). Kaethner (*29*) also evaluated the toxicity of two neem insecticides to *C. septempunctata.* High concentrations (250 and 1000 ppm azadirachtin) were virtually nontoxic to eggs, 2nd instars and adults when they were exposed to dried residues on bean leaves. Toxicity to larvae, however, was evident after exposure to direct sprays.

Lowery and Isman (*30*) found that a neem insecticide caused no reduction in survival of C. undecimpunctata L. larvae after topical exposure. However, exposure of larvae to treated foliage and treated food (aphids) resulted in no adult eclosion.

Banken and Stark (*31*) found that the neem insecticide Neemix was relatively non-toxic to 1st and 4th instars of *C. septempunctata* after direct spray application. LC50's were substantially higher than recommended field application rates. However, in a later study, *C. septempunctata* was found to be more susceptible than indicated by the earlier study. Banken and Stark (*32*) exposed *C. septempunctata* to direct sprays, treated foliage and a pesticide-treated food source, pea aphids. Multiple routes of exposure resulted in greater mortality and effects on egg laying than direct exposure alone. However, the equivalent of 100 ppm azadirachtin, which is a concentration above the recommended field rate, was required to cause a significant effect.

Two ladybeetles, *Cycloneda sanguinea* (L.) and *Harmonia axyridis* (Pallas), were exposed in the laboratory to neem oil (*33*). Significant mortality of *C. sanguinea* larvae occurred after exposure to neem oil as a leaf residue, but not after topical application. However, no negative effect was found in larvae of *H. axyridis*.

Qi et al. (*34*) fed *Harmonia conformis* (Boisduval) the larvae of *Helicoverpa armigera* (Hubner) that had fed on neem. They found that *H. armigera* larvae exposed to 50- and 200-ppm azadirachtin treatments were not toxic to *H. conformis*.

Elzen and James (*35*) evaluated the toxicity of neem oil and azadirachtin to the ladybeetle *Coleomegilla maculata* De Geer. Both of these products exhibited low toxicity to this species.

Based on the results of the above-mentioned studies, it appears that the toxicity of neem to ladybeetles depends upon the formulation being evaluated and the susceptibility of individual species, and thus no generality about the toxicity of neem and ladybeetles can be made.

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Predaceous mites

Stark et al. (*36*) found that a predaceous mite, *Iphisieus degenerans* Berlese, and its prey, the two-spotted spider mite, *Tetranychus urticae* Koch, were equally susceptible to the neem-based insecticide Neemix based on comparisons of the acute LC50. However, when population growth rate was examined, the predator was much more susceptible than its prey. Two spotted spider mite populations were able to maintain a positive growth rate after exposure to the acute LC_{91} of immatures and the LC_{58} of adults. Predator mite populations were declining and headed for extinction when exposed to the acute LC_{57} for immatures and the LC_9 for adults. Thus, in this study neem was not selective and in fact was much more toxic to the predator than to its prey.

The side effects of the neem insecticide Neemark on *Phytoseiulus persimilis* Athias-Henriot, a predator of the twospotted spider mite *T. urticae,* were evaluated by Papaioannou et al. (*37*). Neemmark at 3% and 5% concentrations was highly toxic to immature stages and the adult of *P. persimilis*.

Childers et al. (*38*) compared the residual toxicity of neem oil to the predaceous mite, *Agistemus industani* Gonzalez (Acari: Stigmaeidae). Neem oil 90 EC applied at 46.8 L/ha was found to be highly toxic to this predator.

The susceptibility of the predaceous mite *I. degenerans* to neem oil and azadirachtin was evaluated by Ludwig and Oetting (*39*). Both of these products caused mortality in this species. Similar findings were obtained by Stark et al. (*36*) with the same species.

Cote et al. (*40*) studied the effects of neem oil on *P. persimilis*. Neem oil caused no mortality in *P. persimilis* up to 14 days after initial exposure, indicating that it could be used compatibly with this predator to control *T. urticae*.

Spiders

Several laboratory and field studies on the effects of neem on spiders have been published. Saxena et al. (*41*) found that topical application of 50 μg of a neem seed kernel extract did not affect the spider *Lycosa pseudoannulata* (Boesberger & Strand). Mansour et al. (*42*) determined that 2.5% extracts of neem seed prepared with various solvents were non-toxic to the spider *Chiracanthium mildei* L. Koch. However, residues from 4% extracts with pentane, acetone, and ethanol resulted in 71, 54, and 33% mortality, respectively. Stark (*43*) found that applications of Margosan-O had no significant effect on spiders inhabiting turf grass.

Other predators

Macrolophus caliginosus Wagner, a mirid hemipteran, was exposed to three neem pesticides, Neem-Amin EC, Stardoor and B.P. 20/S (*44*). All three products were harmful to first instar nymphs exposed to fresh dry residues on glass plates, resulting in LD50 values much lower than the maximum recommended use rate.

Effects on Other Non-Target Organisms

Bees

Schmutterer and Holst (*45*) conducted field studies to evaluate the impact of neem on honey bees foraging on neem-treated mustard and rape. They found that large honey bee colonies were unaffected by the neem treatments while low numbers of bees were killed in smaller colonies. Bunsen (*46*) conducted both laboratory and field studies on the effects of neem and honey bees and found that only direct contact of larvae with neem resulted in mortality.

Earthworms and Beneficial Nematodes

Neem soil treatments were found to increase *Eisenia fetida* (Savigny) weight, survival and reproduction (*47*).

Stark (*48*) investigated the effects of the neem insecticide Margosan-O on the entomopathogenic nematode species *Steinernema carpocapsae* (Steiner), *S. feltiae* (Filipjev) and *S. glaseri* (Steiner). Margosan-O was toxic to all three

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species but only at concentrations higher than the recommended field rate (20 ppm). Infectivity was only affected at concentrations greater than 200 ppm azadirachtin. In an earlier study, Rovesti and Deseö (*49*) found that a crude neem extract was toxic to five species of entomopathogenic nematodes including the three species evaluated by Stark (*48*). Hussaini et al. (*50*) explored the compatibility of neem and two strains each of the entomopathogenic nematodes *Steinernema bicornutu* Tallosi, Peters & Ehlers and *Heterorhabditis indic*a Poinar, Kanunakar, and David. Neem was not toxic to *S. bicornutum* but was toxic to one strain of *H. indica.* The effects of neem on the entomopathogenic nematode *S. feltiae* was evaluated by Krishnayya and Grewal (*51*). Neem oil had no effect on the viability and virulence of *S. feltiae.*

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Aquatic Invertebrates

Several laboratory studies have been conducted to examine the toxicity of various neem pesticides on aquatic invertebrates, particularly water fleas (*Daphnia*). For example, Saucke and Schmutterer (*9*) estimated the LC50 for *Daphnia magna* Straus at 0.19 mg/L while Scott and Kaushik (*52*) estimated an LC50 of 125 mg/L for the same species. Stark (*12*) evaluated the toxicity of three commercially produced neem insecticides, Neemiz, Azatin and RH-9999 (a wettable powder containing 20% 22, 23-dihydro-azadirachtin) to the water flea *D. pulex* (Leydig). He found no significant difference between Neemix and Azatin in terms of LC50, but RH-999 was significantly less toxic than the other two products.

Fish

Several studies involving estimates of toxicity of neem to various fish species have been published. Attri and Prasad (*53*) studied the toxicity of neem oil extract to *Gambusia* sp. in the laboratory. The extract was nontoxic at 0.005% but caused 100 % mortality at 0.4%. Margraf (*54*) applied neem oil (10-100 mg/L) to rice paddy fields in the Philippines and found no negative effect on the fish *Misgurnus anguillicaudatus* (Cantor). Wan et al. (*55*) estimated the 96 h LC50 of pure azadirachtin to juvenile coho and Chinook salmon to be approximately 4 mg/l. The acute toxicity of water extracts of bark of the Neem tree was evaluated in the cichlid *Tilapia zilli* Gerv by Omoregie and Okpanachi (*56*). The 96 h LC50 was estimated to be 6.03 mg/l. Exposure to the neem extract also increased opercular ventilation rates. Prior to death, darkening of the exposed fish, erratic swimming, and respiratory distress occurred.

The acute toxicity of neem to Indian carp was evaluated by Das et al. (*57*). Fingerlings of Rohu (*Labeo rohita* [Hamilton]), Catla (*Catla catla* [Hamilton]), and Mrigal (*Cirrhinus mrigala* [Hamilton]) carp were exposed to limnoids of neem, and the 96 h LC50's were estimated to be 2.36, 2.04, and 2.78 ppm, respectively. In summary, toxicity studies with fish indicate that some species may be susceptible to neem while others are not.

Field Studies

Aquatic Field Studies

Several field studies on the effects of neem insecticides on aquatic organisms have been conducted. See Thompson and Kreutzweiser (Chapter 18 of this publication) for a more detailed discussion of field experiments with neem. Dunkel and Richards (*58*) studied the effects of a neem insecticide on nontarget stream insects and found that these species may be vulnerable to neem insecticides at the expected environmental concentration (EEC) of 0.035 mg/l. However, a field study conducted by Kreutweizer et al. (*59*) indicated that the neem-based insecticide Neemix caused significant changes to an aquatic community, but only at concentrations much higher than the EEC (0.035 mg/l). These conflicting results indicate a need for further research on the aquatic ecotoxicological effects of neem.

Terrestrial Field Studies

A comparison of the impact of the neem insecticide, Margosan-O and the synthetic organophosphorous insecticide, chlorpyrifos on invertebrates inhabiting a turf grass ecosystem was conducted by Stark (*5*). Margosan-O had much less effect on most of the invertebrates studied compared to chlorpyrifos. However, certain groups of invertebrates, particularly the Oribatid mites were more susceptible to neem than to chlorpyrifos. The sminthurid and non-sminthurid Collembola were less susceptible to Margosan-O than to chlorpyrifos but their populations were significantly reduced compared to the control. Chorpyrifos, but not Margosan-O, significantly reduced populations of non-oribatid mites and spiders. In general, Margosan-O was less detrimental than chlorpyrifos to most of the organisms studied.

Ma et al. (*60*) conducted a field study to control *Helicoverpa* spp. in cotton. Moderate rate-dependent control was obtained in plots treated with neem seed extracts containing azadirachtin at rates of 30, 60 and 90 g/ha. Several predators, including lady beetles, lacewings, spiders and predatory bugs, were not affected by neem.

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Conclusions

Based on the studies reviewed here it appears that some nontarget organisms are susceptible to neem pesticides while other species are not very susceptible. However, much of the data suggest that insecticides derived from the neem tree are less likely to cause substantial environmental damage than synthetic insecticides. Because the active ingredients in neem are poisons and some species are very susceptible to these products, they should be used cautiously. Future studies designed to investigate the contribution of various neem liminoids and neem oil to toxicity of nontarget organisms should be conducted so that specialized neem products can be developed to target certain pests while sparing their natural enemies. Additionally, the contribution to toxicity of individual adjuvants that are components of various neem formulations should be determined so that they may be avoided when designing new neem pesticides.

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